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B. T. GALLOWAY, *Chief of Bureau.*

# A KNOT OF CITRUS TREES CAUSED BY SPHAEROPSIS TUMEFACIENS.

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# LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
*Washington, D. C., February 29, 1912.*

Sir: I have the honor to transmit herewith a manuscript entitled "A Knot of Citrus Trees Caused by Sphaeropsis Tumefaciens," by Miss Florence Hedges, Assistant Pathologist in the Laboratory of Plant Pathology, and Mr. L. S. Tenny, formerly Assistant in Plant Pathology, and to recommend its publication as Bulletin No. 247 of the series of this Bureau.

This paper deals with an infectious disease of citrus trees which has caused extensive losses in Jamaica, British West Indies. The same or a very similar trouble has recently been discovered in the United States.

The investigations reported herein have extended through a period of six years, during which time the cause of the disease has been established beyond a doubt by means of repeated inoculations, reisolations, and reinoculations.

The authors desire to acknowledge the courtesies shown them and assistance rendered by Hon. William Harris and Hon. William Fawcett, director and former director, respectively, of Public Gardens and Plantations, Kingston, Jamaica; Hon. William Cradwick, Department of Public Gardens and Plantations, Jamaica; Hon. Mr. Cousins, in charge of the Government laboratory; the late Mr. J. A. Stephens, of Radnor; Rev. J. G. Hall, Mr. George Costa, Mr. Edwards, and Mr. Malcolm, Jamaica, British West Indies.

Respectfully,

B. T. Galloway,
*Chief of Bureau.*

Hon. James Wilson,
*Secretary of Agriculture.*

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NOTE.—The photographs and most of the drawings illustrating this bulletin are the work of Mr. James F. Brewer, artist, Laboratory of Plant Pathology.

B. P. I.—733.

# A KNOT OF CITRUS TREES CAUSED BY SPHAEROPSIS TUMEFACIENS.

## INTRODUCTION.

The fungus under discussion in this paper was first isolated in 1904 from knots on lime trees from Jamaica (Pl. I). Later it was also obtained from orange-tree knots from the same island (Pl. II). Repeated inoculations with both strains and reisolation of the fungus from the knots so produced have proved conclusively its parasitism. Previous to 1911 no such disease, so far as the writers know, had been observed in the United States, but in the spring of that year a knot similar, if not identical, was received from Florida.[1] No reference to the disease, either in this country or elsewhere, has been found in literature. A short preliminary account of the disease with a description of the fungus has been published.[2]

In 1906 one of the writers (Hedges) visited several of the larger Jamaican "pens" (estates) on which limes and oranges were cultivated, for the purpose of studying the disease in the field. It was found, however, that although in previous years the disease had caused much trouble in the northern and western parts of the island, the majority of the growers had been able to keep it under control by cutting and burning and no longer considered it very serious.

---

[1] In May, 1911, a knot on a lime twig was received from Quay, Fla. It resembled the Jamaican knot and was thought to be due to the same cause. In response to inquiry the sender wrote as follows: "I do not find any other growth than the piece which you have. This tree was on the place when I purchased it in 1908. I judge the tree to be about eight years of age. It has always been healthy until this last year, when it showed disease, as evidenced by numerous dead branches. These I am pruning out."

Mycelium resembling that of *Sphaeropsis tumefaciens* Hedges was present in the bark and wood of the knot and also in black streaks in the wood of the stem below the knot. The fungus was isolated from both knot and stem by the method employed for the Jamaican knot.

Inoculations were made on lime twigs and branches with a freshly isolated culture (13 days), and these have been under observation for seven months as this bulletin goes to press. Three of the inoculated shoots were killed, and although no real knots have formed on the others, considerable tumor tissue has been produced and mycelium is present in the tissues. There has been a large production of gum in every case.

On corn meal, however, the only medium on which the fungus has fruited, the spores are larger and thicker walled than any of those of *Sphaeropsis tumefaciens* that have been observed thus far. They measure 28 to 36 $\mu$ by 11 to 16 $\mu$, and when they become colored they are a decided brown instead of yellow. By no means do they always become brown, however. No 2-celled spores have been observed.

[2] Hedges, Florence. Sphaeropsis Tumefaciens, Nov. Sp., the Cause of the Lime and Orange Knot. Phytopathology, vol. 1, no. 2, 1911, pp. 63–65.

Mr. George Costa, of Orange Valley, St. Anns, who had watched the disease for several years and whose groves had suffered extensively from it, made the following statements regarding it:

1. It attacks trees of all ages, old as well as young wood.
2. Knots appear in any season, regardless of weather conditions.
3. The disease spreads from one tree to another.
4. If an affected tree is cut down, the young shoots coming up from the roots develop knots.
5. From the knots an abnormal number of branches spring (witches'-brooms).
6. An affected tree has a whole series of knots extending up the branches, not simply a few isolated ones.
7. An affected tree may live about 18 months,[1] but always dies prematurely as a result of the disease.
8. Trees growing in the so-called "red dirt," which is rich in iron, are more often attacked.

At the time of the writer's visit there were no badly diseased trees in Orange Valley. The disease, which had formerly been very prevalent there, seemed to have been held in check by the severe pruning. There were scattered knots, however, and specimens of these were brought to Washington.

## DESCRIPTION OF THE KNOTS.

The knots vary in size from three-eighths of an inch to 2 or 3 inches in diameter. Generally they are approximately round, but in the earlier stages before they surround the stem they have a broad, flattened base and may be somewhat elongated, the longer axis being parallel to that of the infected branch. In some cases, instead of forming a well-defined gall the disease produces an eruption extending several inches along the limb. This consists of slightly raised and variously fissured portions.

At first the incipient knot is covered by the bark, but after some months this usually dies and falls away, exposing the enlarged woody tissues. In the early stages of growth the surface of the knot (the bark) is light colored and rather smooth. With age the surface becomes darker and more or less fissured, and an old gall divested of its bark is often almost black, rough, knotty, and deeply furrowed. When the growth is rapid, however, the malformation may reach a large size and the bark surface still be intact, smooth, and light in color.

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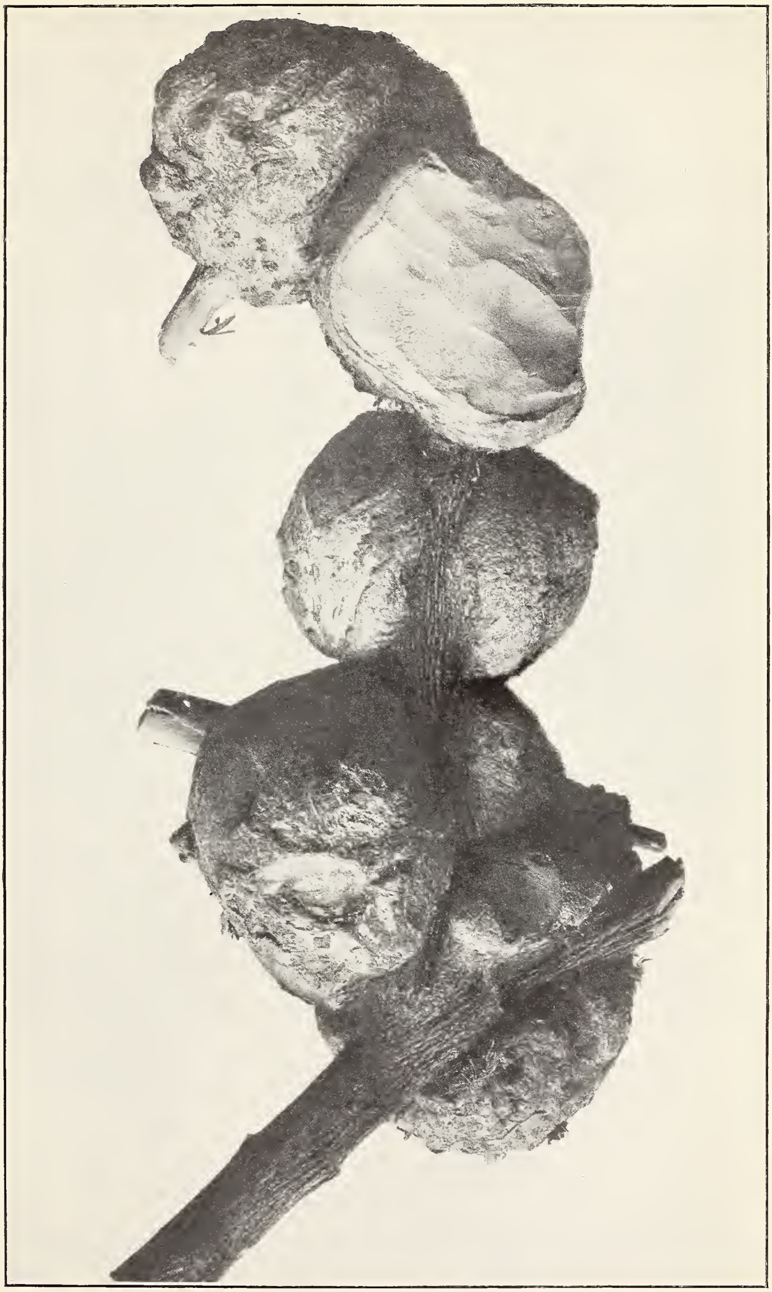
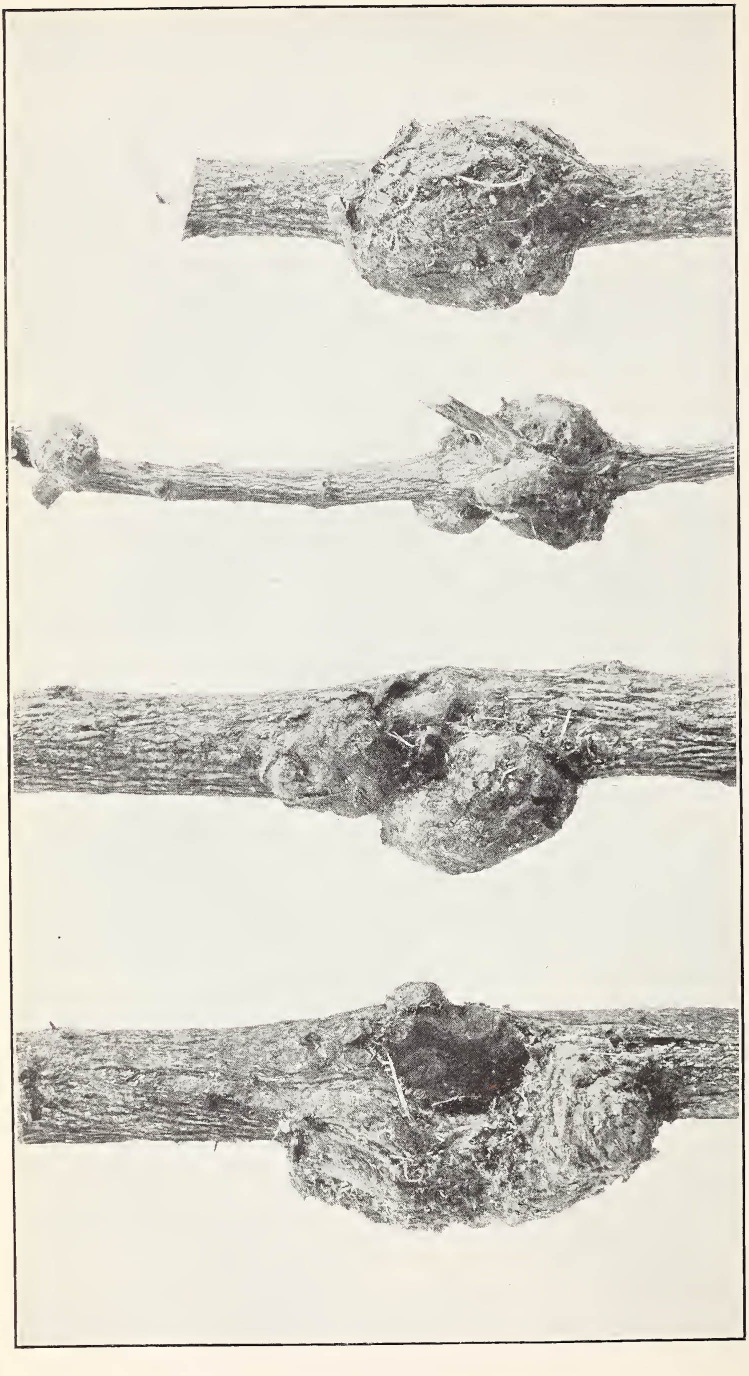
[1] Trees inoculated in the greenhouse in Washington are alive at the end of five years, although in many cases the portions of the branches above the knots have died.



LIME KNOTS RECEIVED FROM JAMAICA IN 1904, ONE OF THEM PARED AWAY TO SHOW THE THIN BARK AND VERY HARD WOODY STRUCTURE.

(Natural size.)



ORANGE KNOTS RECEIVED JANUARY 2, 1909, FROM JAMAICA.

(About natural size.)

The surface of the knot is usually quite different in texture from that of the healthy stem; the firm greenish bark is replaced by a softer, more or less crumbling surface, cutting easily with a knife. The interior of the knot, however, is very hard and compact, being composed of firm woody tissues. The outer layer of the knot (modified bark) is about the thickness of ordinary bark, i. e., the knot grows chiefly by additions to the woody, not to the cortical tissues. The color of the interior of the knot may be similar to that of healthy wood or it may be more or less streaked with black. The knots are attached by a broad base and can not be broken off. As they grow they extend laterally and may ultimately girdle the branch.

The first indication of infection is a slight swelling of the branch. As this increases in size the bark, which at this stage covers the young knot, becomes lighter in color and is noticeably cracked and has a corklike appearance. Growth under greenhouse conditions in Washington is slow, several to many months being necessary for the production of knots 1 to 3 inches in diameter. When many knots appear upon a branch the single ones may be smaller. In the artificial infections in the Department greenhouses it has taken from five months to two years for the knots to girdle the branch and to become 1 to 2 inches in diameter, but our temperatures were considerably lower and the moisture less than in Jamaica. By the time the stem is girdled all of that part of the branch above the knot is usually dead or dying. (Pl. III.) When a tree contains numerous infections, therefore, a large portion of it dies.

A striking characteristic of the lime knot is the growth of a large number of new shoots from the knots, forming typical witches'-brooms (Pl. IV, figs. 1, 2). This did not always take place in the greenhouse inoculations, although it frequently did. Sometimes there were only one or two shoots from the knot, but more often the number was greater, and knots have been observed which bore 30 or 40 small shoots. These shoots grow rapidly, and some of them soon reach a height of several feet with few, if any, branches, and often bear knots. Usually after some months all of these shoots die.

In case the tree has a large number of infections the death of parts beyond the encircling knots results in the loss of the greater part of the tree, and what remains is so weakened as to be of no commercial value. As the disease develops, secondary knots form on the main branches and trunk (Pls. V and VI), often extending to the ground, and the death of the entire tree is the ultimate result.

Gum frequently oozes from the knots, and gum pockets are of common occurrence in the infected wood, but this gummosis is not peculiar to the lime knot.

## CAUSE OF THE DISEASE.

This disease is of fungous origin. The facts supporting this statement are as follows:

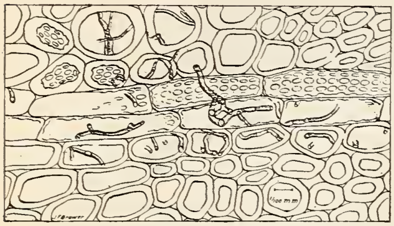


FIG. 1.—Cross section of a lime knot, showing location of hyphæ in wood cells and medullary ray. Stained with haematoxylin and safranin.

A microscopical examination of the knots and of the stem above and below the tumors revealed the presence of mycelium in abundance in the cells of all the tissues. It was particularly abundant in the cortex, which was almost a solid mass of black mycelium in some of the orange knots. The wood also contained quantities of it (figs. 1 and 2), and the pith was likewise invaded (fig. 3). Later it was observed that large rapidly growing knots as a rule contained much less mycelium than the smaller slow-growing ones, and usually the greater part of the hyphæ in the former were hyalin. In the young rapidly growing tissues of buds growing out of knots the mycelium is confined to the intercellular spaces (fig. 4).

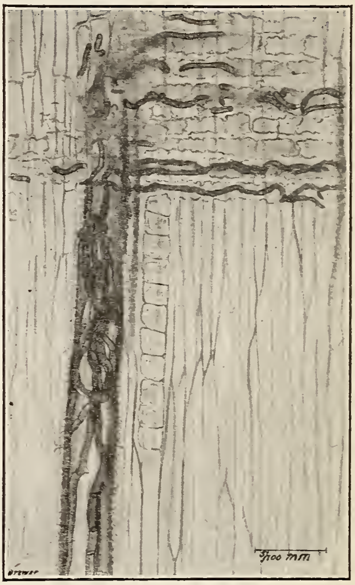
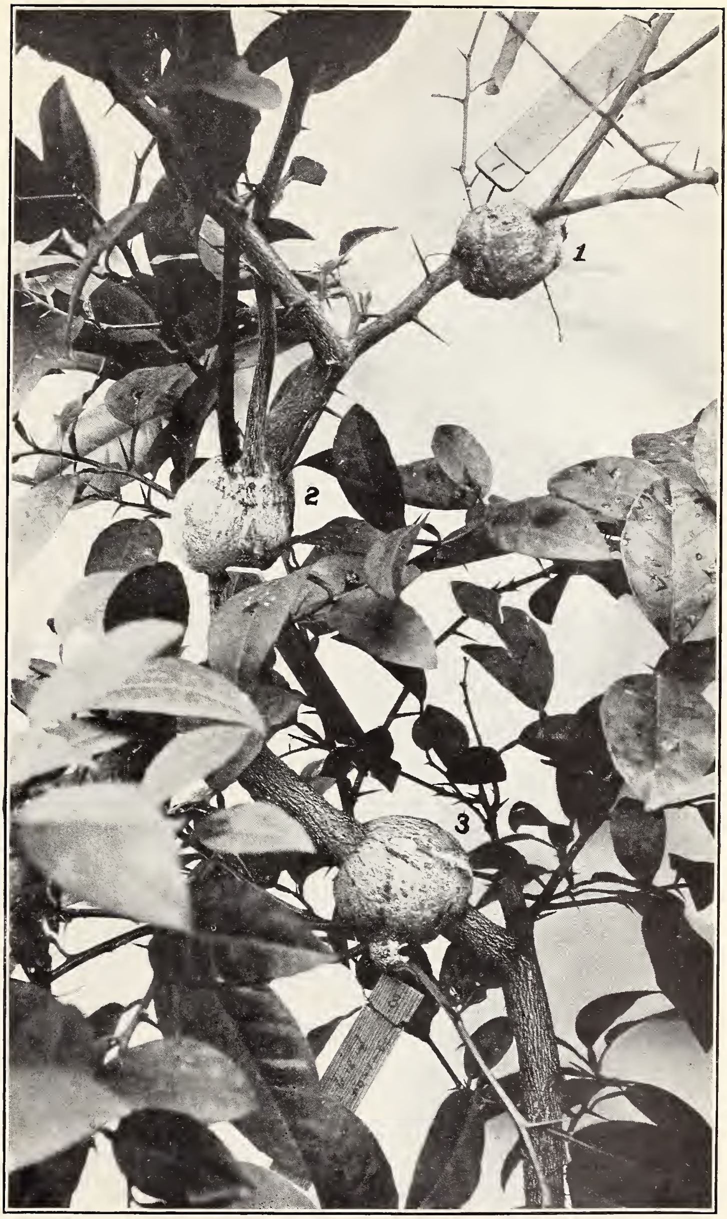


FIG. 2.—Radial section of stem above a knot on orange, showing mycelium of *Sphaeropsis tumefaciens* traversing the wood in various directions. This section was made from a natural infection at a distance of 1 inch above a knot; shows fungus in vessel and medullary ray. Reduced from a camera drawing made with Zeiss 8 mm. objective and No. 12 compensating ocular.

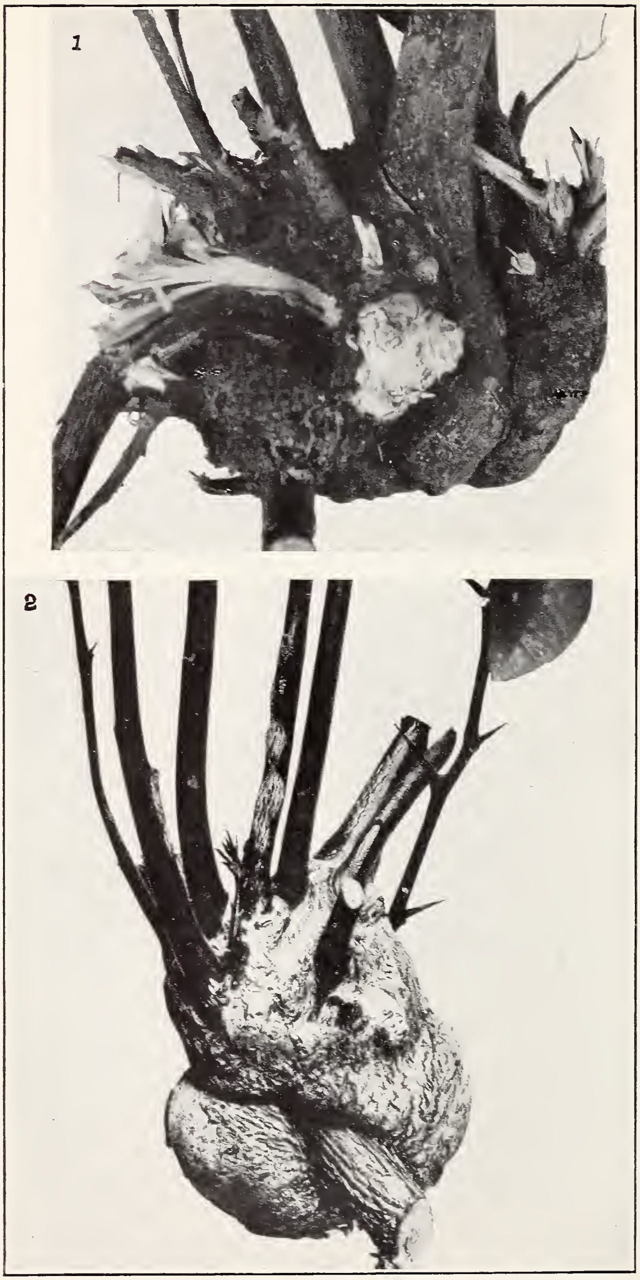
The first isolation of the fungus was made by cutting, under sterile conditions, small pieces of the wood from the interior of the knot and transferring them to tubes of sterile water and beef bouillon. In three or four days pure cultures of the fungus had developed in each tube inoculated, the mycelium pushing out of the interior tissues. Isolations have also been made from the stem above and below the knots. In one instance a pure culture was obtained from the interior of a branch coming out 9 inches above a knot on the main stem.

Using the fungus thus isolated, inoculations made on healthy young trees in the Department greenhouses produced numerous knots resembling in every respect those received from Jamaica. (Pls. III to VI.) From these artificially produced knots the fungus was again isolated and when pure cultures of it were inserted under the bark of healthy



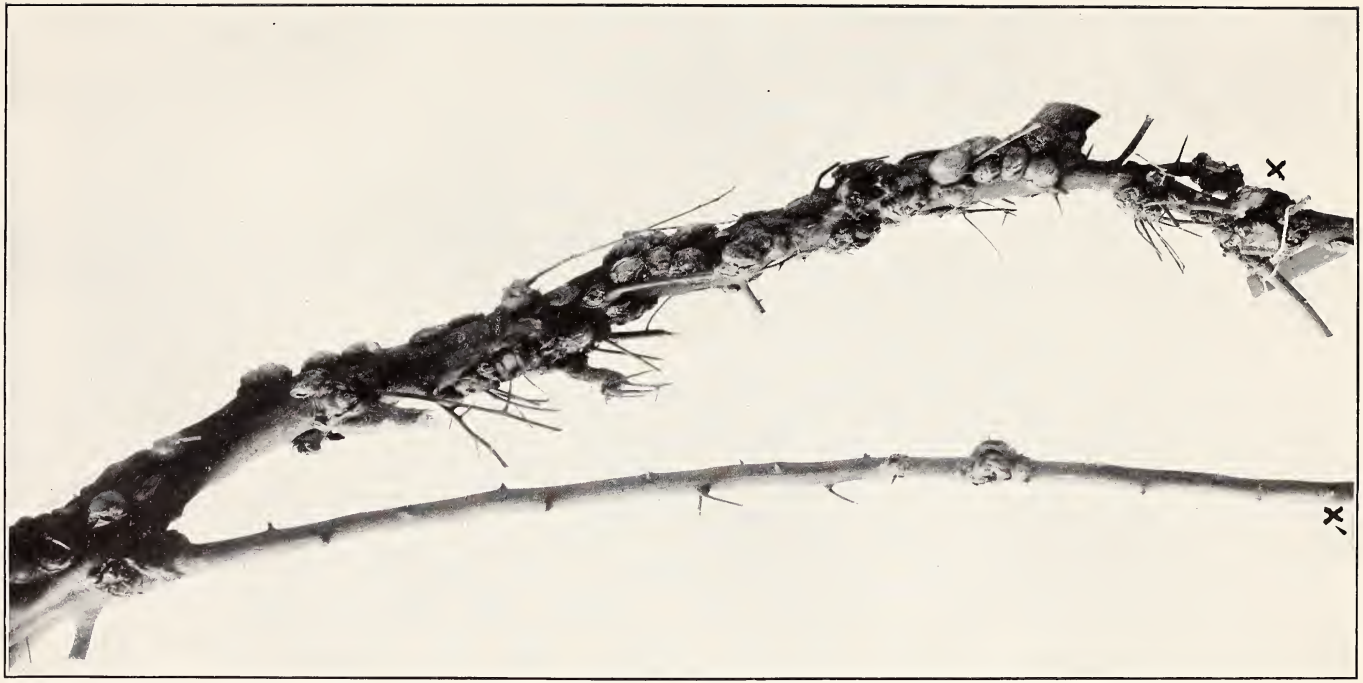
THE FIRST LIME KNOTS PRODUCED BY PURE-CULTURE INOCULATIONS WITH SPHAEROPSIS TUMEFACIENS OBTAINED FROM THE KNOTS SHOWN IN PLATE I.

The inoculated branch is much diminished in size between inoculations Nos. 1 and 2, and is dead above inoculation No. 1. Tree I, inoculations Nos. 1, 2, and 3, December 1, 1904. Photographed March 31, 1906. Hothouse, Washington, D. C. (About two-fifths natural size.)



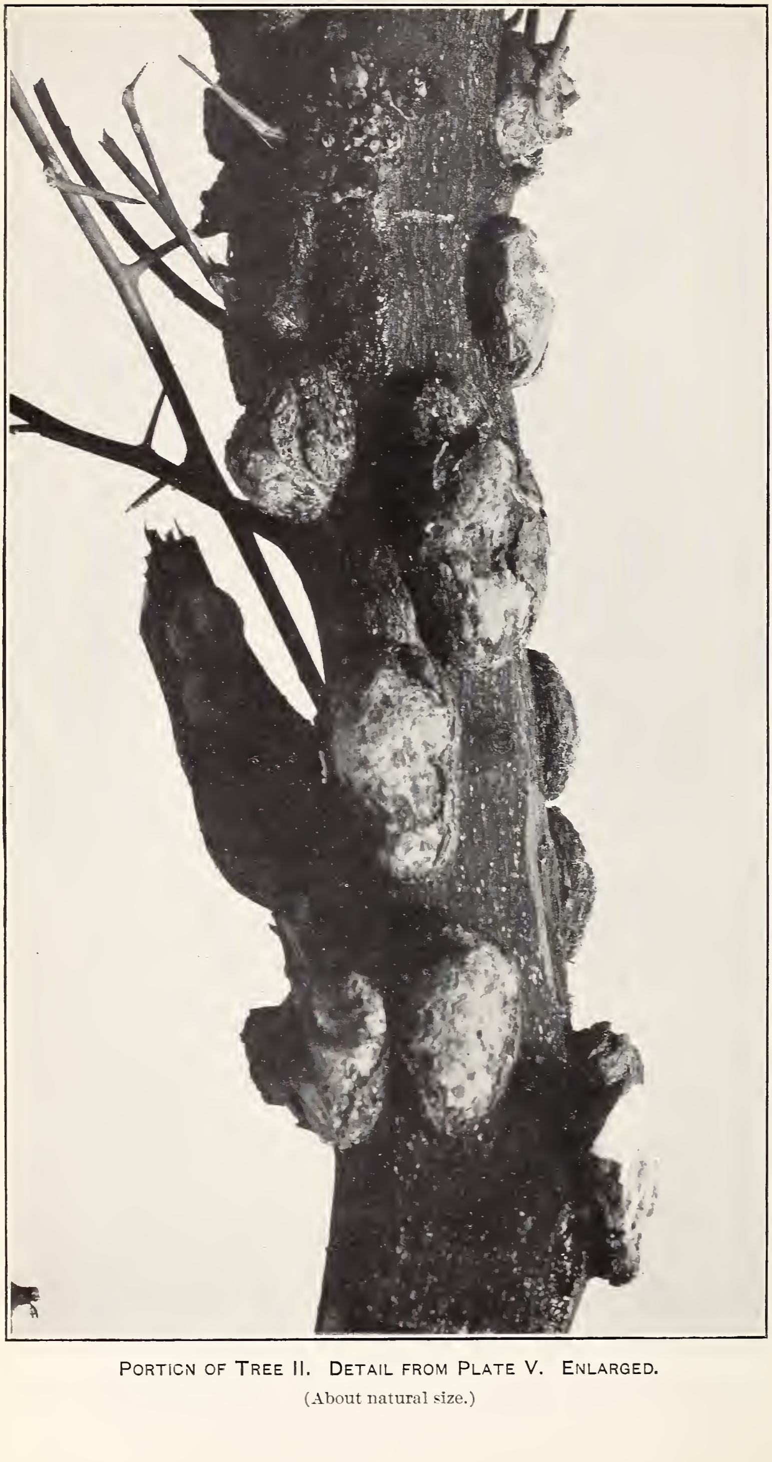
WITCHES'-BROOMS PRODUCED ON LIME TREES BY SPHAEROPSIS TUMEFACIENS.

FIG. 1.—Witches'-broom brought from Jamaica in July, 1906. FIG. 2.—Witches'-broom produced by inoculation No. 4, December 1, 1904; eruption extending up the stem in one of the shoots. Photographed December 1, 1905. (Natural size.)



PORTION OF TREE II INOCULATED AT X (INOCULATION NO. 5), DECEMBER 1, 1904, SHOWING NUMEROUS SECONDARY KNOTS.

The fungus was traced through the stem to X′, a distance of 5 feet from the point of inoculation. The infection covers a period of 3 years and 7 months. (About one-fifth natural size.)



Portion of Tree II. Detail from Plate V. Enlarged.

(About natural size.)

lime trees more knots resulted. The number of successful inoculations, reisolations, and reinoculations proves beyond a doubt that this fungus is the cause of the disease.

## ISOLATION OF THE FUNGUS.

The fungus is easily isolated from the knots. It is preferable to use fresh young knots for this purpose, although the original isolation was made from knots about 3 inches in diameter. In the various isolations made by the writers the knots were thoroughly washed and soaked in mercuric chlorid (1 to 1,000) from three minutes to one hour before cutting out portions of the interior as material for cultures. Three minutes were found to be sufficient. Dipping in alcohol before transferring to mercuric chlorid insures a thorough wetting of all portions by the latter, and this method was usually followed. If the surface of the knot was very rough or cracked, this part was cut away previous to the soaking in mercuric chlorid. After a thorough rinsing in sterile water, the knots were cut in two and portions of the inner tissue were cut out with a sterile knife and dropped into tubes of culture media, preferably sterile water. Pure cultures were obtained in two to three days by this method.

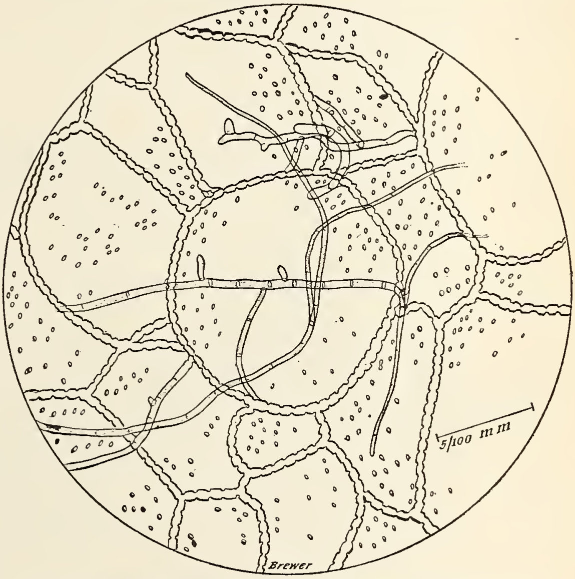


FIG. 3.—Cross section of lime stem, 2½ inches above a knot, showing mycelium in the pith.

The fungus has also been seen many times in the stem between the knots and has been isolated therefrom. (Pl. VII, fig. 6.)

## DESCRIPTION OF THE FUNGUS SPHAEROPSIS TUMEFACIENS.

*Mycelium.*—Within the host the fungus occurs as a branching, much-septate mycelium, consisting of colorless or brown hyphæ. The colorless young filaments soon change to a pale brown, which at length deepens into a shade so dark that in a mass the mycelium looks black. It is the massing of these old hyphæ which imparts the black color to portions of some of the knots. The color is located in the cell walls. Both the hyalin and brown hyphæ vary a great deal in diameter. (Pl. VIII, fig. 9.) In measurements made of the brown hyphæ in one of the knots the diameter ranged from 2 to 4 μ, and that of the hyalin hyphæ in the same knot from 0.4 to 3.5 μ. Very much coarser hyphæ commonly occur in cultures. (Pl. VIII, fig. 10.) The walls of the hyphæ may be straight or often quite irregular in outline. The tips of the young hyphæ growing from infected wood sterilized externally and put into tubes of sterile water are commonly somewhat swollen. In cultures the very young hyphæ are sparingly septate, but in a few days to many weeks there are usually many coarse brown filaments, which are divided into approximately isodiametric cells, frequently with bulging lateral walls. (Pl. VIII, fig. 9.) Often on artificial media, especially in old cultures, hyphæ occur in which the walls are thickly beset with warts. (Pl. VIII, fig. 8, and Pl. IX, fig. 5.) Sometimes these warts are 2 to 4 μ thick. (See lime-wood cylinders, p. 31.) Fused hyphæ are also common in old cultures. (Pl. VIII, fig. 7.)

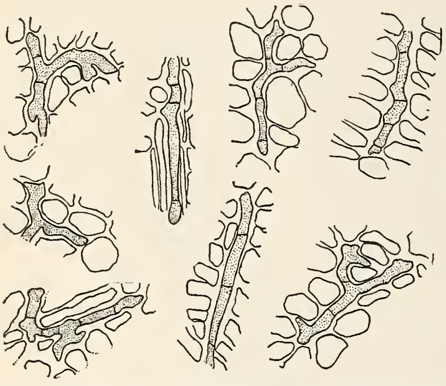
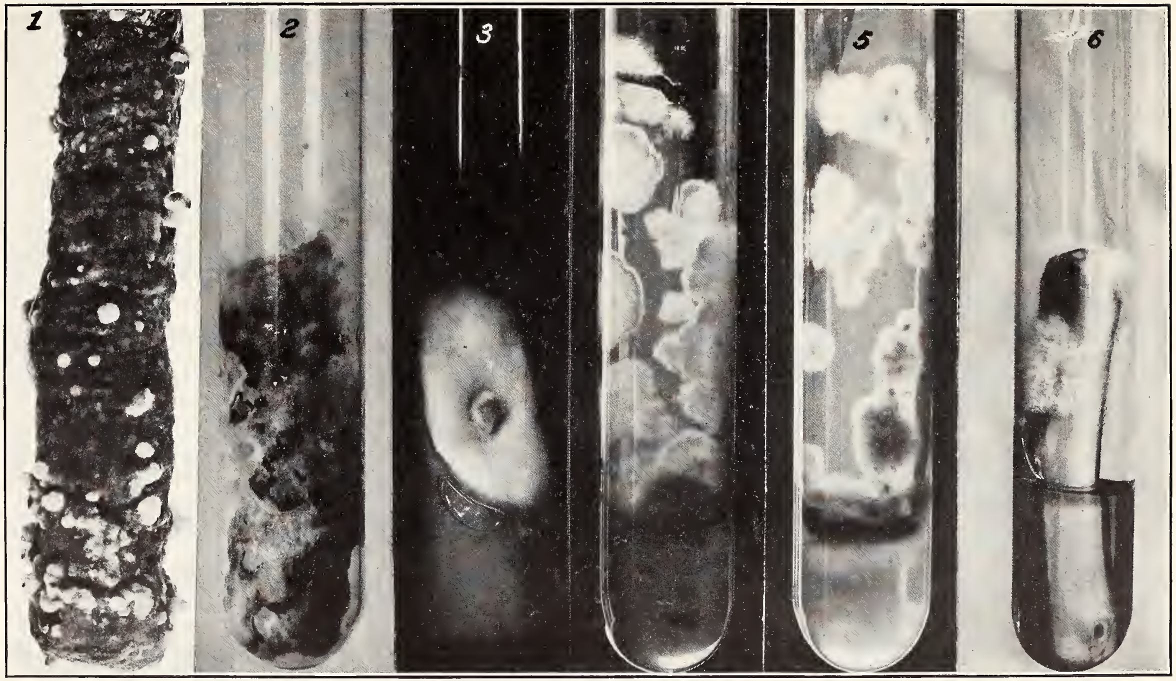


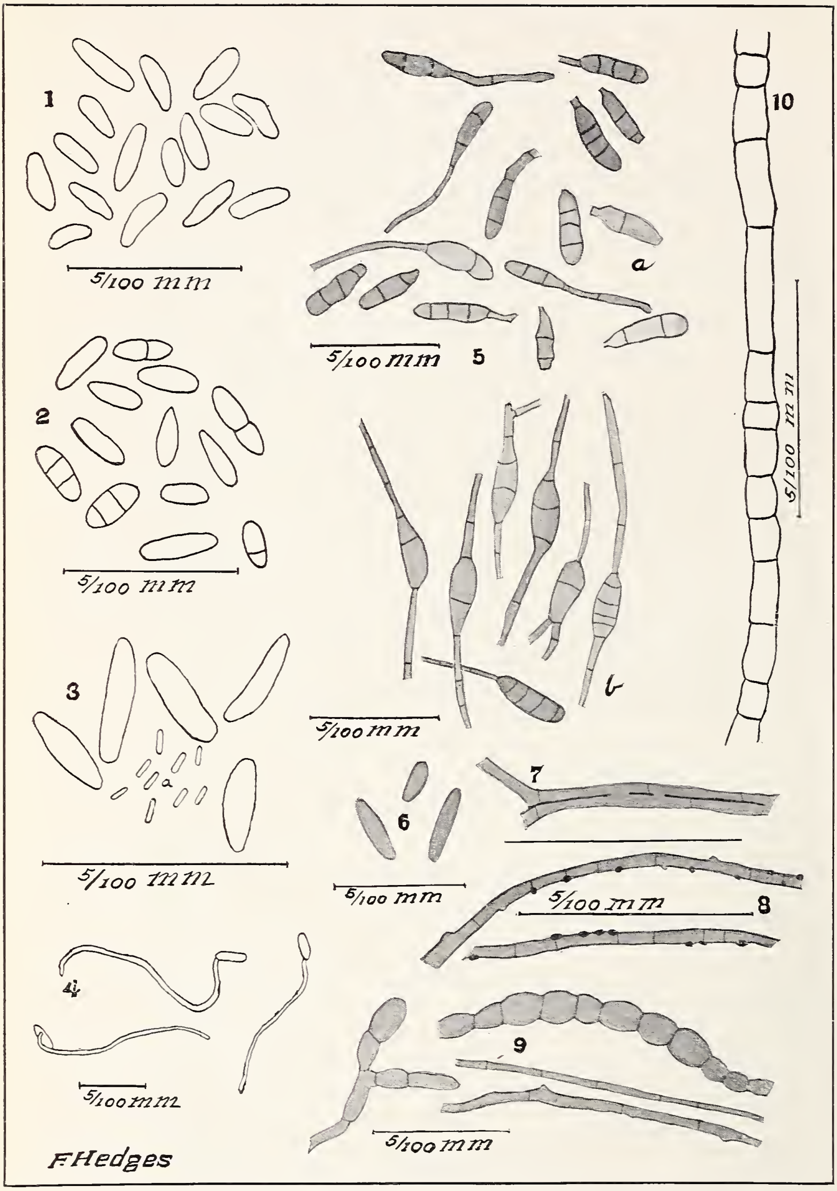
FIG. 4.—Mycelium in intercellular spaces in young, growing tissues. Section made from a bud growing out of a knot.

*Pycnidia.*—Until the summer of 1909 the fungus was never observed fruiting on the host, either in natural infections or those produced artificially in the greenhouse, nor had it been found producing spores in culture media previous to the spring of that year, although it had been tried in a great variety of media. (See corn-meal cultures, orange strain, p. 19.) However, masses of intertwining hyphæ suggestive of pycnidia in the process of formation (Pl. IX, fig. 6) frequently had been observed. (See prune and beef agars, pp. 22 and 23.) From the first corn-meal cultures containing pycnidia (orange strain) a vigorously fruiting strain has developed. A second (nonfruiting) strain isolated from the orange, however, has never produced spores in cultures, so far as observed, but produces them abundantly on the host. Later a second fruiting strain was isolated from a lime knot produced by inoculation with the fruiting orange strain. The lime strain has thus far fruited very rarely. Spores have been found only in some of the more recent inoculations and in very old cultures (18 months to several years old) and then (i. e., in the cultures) only in very small numbers and not in all the cultures in the same



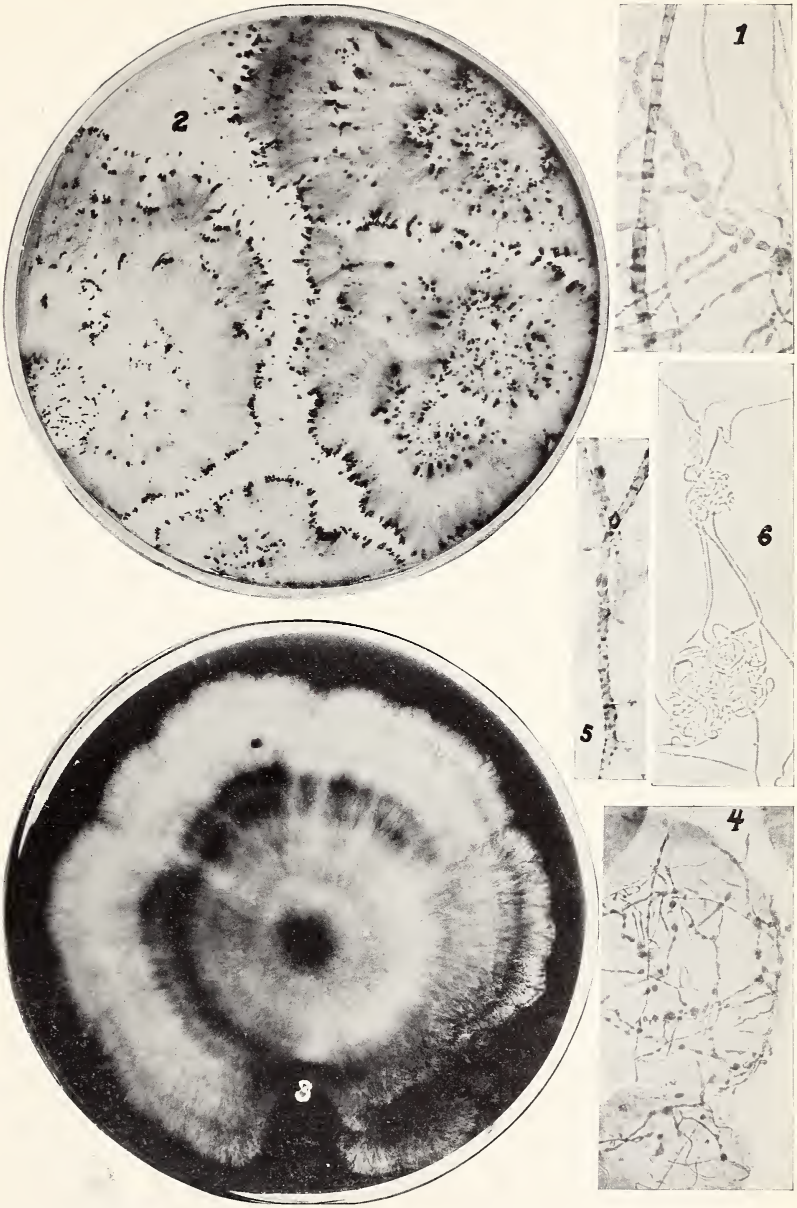
TEST-TUBE CULTURES OF SPHAEROPSIS TUMEFACIENS.

FIG. 1.—Growth on sterilized lime wood, showing the small spherical masses of pseudotissue; 6-weeks-old culture; used for inoculations Nos. 1–6 of December 1, 1904. FIG. 2.—Potato culture 5 weeks old showing stroma. FIG. 3.—Potato culture 3 days old. FIG. 4.—Neutral potato-agar culture 11 days old (lime strain). FIG. 5.—Neutral potato-agar culture 11 days old (orange strain). FIG. 6.—Infected lime stem in sterile water, showing presence of the fungus in the stem between tumors. Surface sterilized by soaking 5 minutes in mercuric chlorid 1 to 1,000. Photographed at the end of 8 days.



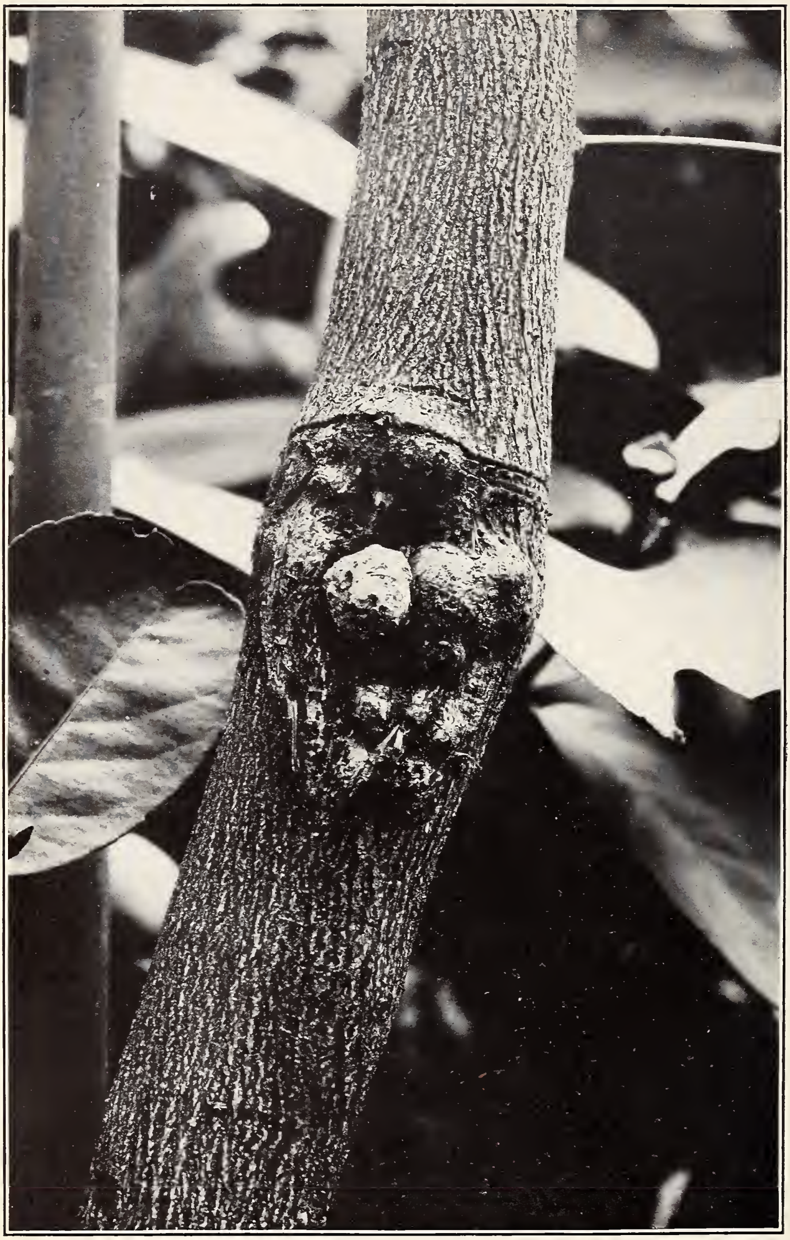
MYCELIUM AND SPORES OF SPHAEROPSIS TUMEFACIENS.

FIGS. 1 and 2.—Pycnospores (orange strain). FIG. 1.—From lime (artificial infection). FIG. 2.—From lemon (raw fruit). FIG. 3.—Pycnospores and spermatia from lime 3 weeks after inoculation (lime strain). FIG. 4.—Germinating pycnospores in potato-agar plate culture, 24 hours old. FIG. 5.—Brown septate bodies. (*a*) Apple (raw fruit, 18 days). (*b*) Steamed potato (6 weeks). FIG. 6.—Yellowish pycnospores on lime 3 weeks after inoculation (orange strain). FIGS. 7, 8, and 10.—Mycelium from potato-agar culture 8 months old. FIG. 9.—Mycelium from corn-meal flask culture 5½ months old. FIG. 10.—Coarse hyalin hyphæ from potato-agar culture 8 months old.



SPHAEROPSIS TUMEFACIENS ON CULTURE MEDIA.

FIG. 1.—Photomicrograph of swollen hyalin cells in beef-agar plate, 4 weeks old. FIG. 2.—Potato-agar plate, 3 weeks old showing pycnidia. FIG. 3.—Colony 10 days old, on prune agar; inoculation made centrally with a fragment of mycelium. FIG. 4.—Photomicrograph of a fragment of potato cylinder from a culture 3 weeks old, showing the penetration of the cells by the fungus and the nearly complete absence of starch. FIG. 5.—Photomicrograph of mycelium from a beef-agar slant 8 days old, showing protuberances on the hyphæ. FIG. 6.—Photomicrograph of interwoven hyphæ in beef-agar plate, 4 weeks old.



LEMON TREE INOCULATED IN JULY, 1908, BY INSERTING SMALL FRAGMENTS OF FRESH LIME KNOTS (ARTIFICIALLY PRODUCED IN HOTHOUSE) INTO T-SHAPED CUTS UNDER THE BARK. PHOTOGRAPHED JUNE 16, 1909.

(Natural size.)

medium. In the inoculations with the two orange and lime strains on July 21 and 29, 1909, and July 20, 1910 (see pp. 55, 57, 58, and 60), pycnidia have appeared on the host in great numbers (fig. 5). Up to the present time, however, the pycnidia have been found only in the early stages of the disease, when there is very little swelling, i. e., two to four weeks after inoculation, or on old knots (one to two years) which have never developed into good-sized tumors. In the latter case, if the branch beyond the knot is dead, this, too, is sometimes covered with pycnidia.

The pycnidia as they have been observed upon the host (fig. 5) are subglobose, papillate, very dark brown or black, ostiolate, closely crowded together or solitary, with a cellular peridium, and measure 152 to 224 μ by 120 to 192 μ. They are at first covered by the epidermis, sometimes being buried quite deeply in the cortex, but finally they burst through to the surface. They are also occasionally found in the wood. On culture media they are sometimes buried in a stroma (see corn-meal and potato cultures, pp. 20 and 27), in which they occur merely as chambers, separate or confluent, and have no distinct pycnidial wall. Pycnidia in cultures often have short necks.

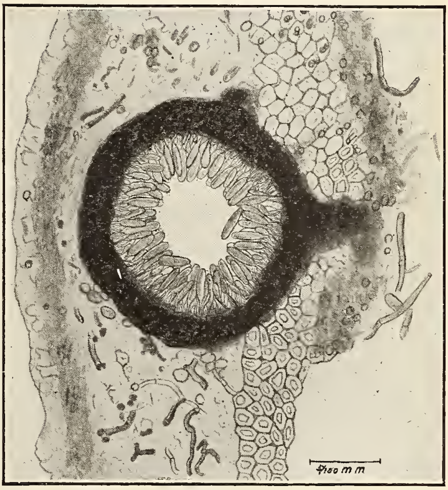


Fig. 5.—Cross section of a lime shoot, showing pycnidium and pycnospores of *Sphaeropsis tumefaciens*, the result of a pure-culture inoculation made July 21, 1909. Stem slightly swollen when collected, August 13, 1909.

*Pycnospores.*—The spores, both on the host and in cultures, are large, colorless or yellowish, oblong or ovoid, rounded at both ends or subacute, sometimes broadly rounded at one end and quite sharply acute at the other, measuring 16 to 32 μ by 6 to 12 μ, rarely 36 by 8 μ in cultures (Pl. VIII, figs. 1, 2, 3, 4, and 6). They are either long and slender (32 by 8 μ) or broad and short (16 by 8 μ), with intergradings. Often they are slightly curved. On the host they are usually continuous, rarely 1-septate, but in cultures on raw apples and lemon, spores with 1 to 2 septa occur quite frequently (Pl. VIII, fig. 2), and occasionally 4-septate spores have been observed. The spores are cut off singly from long or short threadlike basidia and originate as the swollen end of the basidium. The spores are sometimes discharged in cirri in cultures. This has not been observed upon the host.

*Spermatia.*—Some time after the appearance of the pycnidia small, cylindrical, colorless, 1-celled, sporelike bodies, the so-called spermatia, are found both in cultures and on the host (Pl. VIII, fig. 3; see corn-meal cultures of the orange strain, p. 20). These are 4 to 8 $\mu$ by 1 $\mu$ or less, usually 4 to 5 $\mu$ long (measurements from corn meal). They may occur in the same pycnidia as the large ovoid spores or in separate pycnidia, the so-called spermagonia. They are cut off from long slender basidia arranged radially. Their germination has not been observed, nor is there any evidence that they function as true spermatia in De Bary's sense of the term.

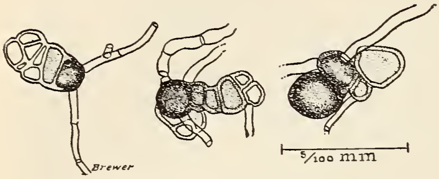


FIG. 6.—Thick-walled, brown chlamydospores from an old pure culture of the lime-knot fungus on banana.

*Chlamydospores.*—No chlamydospores have ever been observed upon the host, but in old cultures on steamed vegetables or fruit large, thick-walled, intercalary, brown, sporelike bodies are sometimes present in abundance. The most striking of those observed were on banana (see p. 27 and fig. 6). On this medium they were often very large, irregular in shape, and many celled. Their germination has not been observed. All gradations of more or less thick-walled, colorless or brown, coarsely granular, swollen cells, occurring singly or in chains, are common in the mycelium on artificial media (fig. 7 and Pl. IX, fig. 1). (See also cultures on carrot cylinders, p. 28.)

On steamed prunes (see p. 29) the mycelial filaments break up into one to several celled fragments, which are found germinating freely in cultures about a week old.

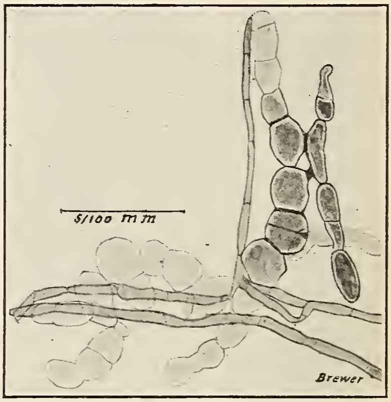


FIG. 7.—Chains of immature chlamydospores of lime-knot fungus from an old pure culture on a carrot cylinder. The majority are colorless.

*Brown septate bodies.*—These brown septate bodies[1] have been observed in potato cultures 6 to 52 days old and on apples (the raw fruit) 18 to 31 days after inoculation with *Sphaeropsis tumefaciens* (Pl. VIII, fig. 5). At first sight one would take them for spores—conidia, chlamydospores, or pycnospores, as the case might be. They are found free or attached to the mycelium as terminal or intercalary bodies, in pockets in the tissues (apple) without pycnidial wall, and, rarely, in pycnidia (apple, steamed potato).

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[1] Observed in one instance on the host (lime) and also on lemon (raw fruit) a month after inoculation.

Although they often occur in quantities, especially on apple, their presence is by no means constant, and even when there are masses of them, comparatively few are detached from the mycelium. Those which are detached sometimes look as though they had been torn off, and sometimes are rounded off in true spore fashion.

The pycnidia in which they have been found have contained none of the true pycnospores and have shown evidences of more or less disorganization. It is believed that these were old pycnidia into which mycelium had grown and produced there the brown septate bodies. The latter are usually devoid of contents, but this is not always the case. Sometimes one cell is filled with granular contents and the others are empty. Occasionally one of the former is rounded off. These septate bodies have never been known to germinate, and it is not believed that they serve in any way as a means of reproduction. They are in all probability only distorted bits of mycelium.

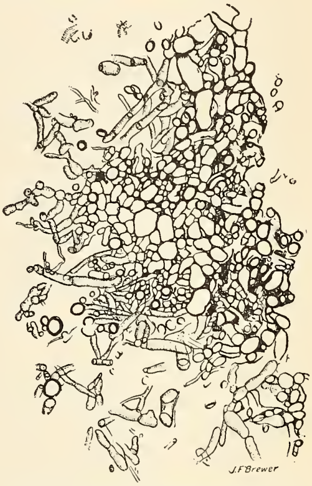

FIG. 8.—Section through one of the cushionlike bodies of *Sphaeropsis tumefaciens* in a pure culture on lime twigs in 2 per cent peptone water. Culture 2 months old; fungus fixed in Carnoy.

*Stroma.*—On many media, e. g., corn meal and potato (see pp. 18 and 26), the fungus produces a thick, black or yellow, tough, wrinkled stroma[1] (Pl. VII, fig. 2), often covered with little cushionlike elevations or stalklike outgrowths (see p. 19) first noticeable as tufts of aerial hyphæ. They are composed of pseudotissue (fig. 8) and often occur on media on which a stroma is not produced (Pl. VII, fig. 1). (See cultures on lime twigs, p. 31.)

## CULTURAL CHARACTERISTICS OF THE FUNGUS.

The fungus grows very well on all of the more commonly used culture media and also on many special media on which it was tried in the hope of obtaining perithecia. The cultures were always kept at room temperature unless otherwise stated. All but the most recent work has been done with cultures descended from isolations from the Jamaican lime knots and in all descriptions this strain is meant unless otherwise stated in the text.

*Steamed corn meal.*—Corn meal in a 100 or a 1,000-c.-c. flask with sufficient water added to make it thoroughly wet after sterilization is an excellent medium for this fungus. Usually the mycelium has covered the surface of the corn meal with a thin layer of creeping hyphæ in five or six days; few, if any, erect hyphæ. At this age the mycelium may be white or grayish white or it may contain a greater or less proportion of dark hyphæ. The same filament may have colorless and dark-colored portions. Very frequently portions, sometimes nearly the whole, of the fungous layer are pale yellow or buff and wet-shining, suggesting in appearance bacterial growth. Very early in the development of a corn-meal culture (not later than the sixth day in any of the cultures made up to date) a tough stromatic layer begins to form. This is usually black, but portions may be pale yellow or buff. It is coated with short grayish white hyphæ, which by the seventh day have formed a soft, gray, velvety surface layer. There is usually an excellent fluffy marginal growth of erect hyphæ 0.5 to 0.75 cm. long growing on the sides of the flask and later forming a stroma there. In cultures in liter flasks there is often a clear amber-colored exudate after about eight or nine days of rapid growth. The surface of the stroma, smooth at first, in about three weeks becomes very uneven and often much wrinkled. Cultures four weeks old frequently have little cushionlike elevations of the stroma, many of which have been examined for pycnidia, though none have been found in cultures of the lime-knot strain up to this time.

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[1] In potato cultures of the fruiting orange strain (q. v.) which are producing spores in abundance this thick, tough stromatic layer is replaced by a much thinner and more crusty layer, which is easily broken or crushed.

The variation in the color of the stroma probably depends upon the amount of moisture, as the yellow or buff stroma has been observed only when the fungus has been grown in liter flasks or when the 100-c.-c. flasks have been supplied with rubber caps. Bits of yellow and black stroma transferred to liter flasks of corn meal produced a stroma the greater part of which in both cases was pale yellow. This was also true of black stroma from a potato-agar culture which was transferred to a 100-c.-c. flask with a rubber cap. In this case the entire stroma was approximately buff colored without a trace of black. The surface hyphæ remained a pure white without the slightest grayish tinge during the entire time it was under observation (31 days). Transfers from this stroma to 100-c.-c. flasks produced the characteristic black stroma and gray surface hyphæ in seven days in the flask without a rubber cap, while in the flask supplied with the cap there was not the slightest trace of black or gray at the end of 12 days, although a good buff-colored stroma had been produced. When the flasks were examined three days later the cap had split and dropped off and the mycelium had a decided gray tinge. In two 100-c.-c. flask corn-meal cultures inoculated with black stroma from a potato culture there was a very slight grayish tinge in both surface hyphæ and stroma in seven days in the flask with the rubber cap, but the difference between this flask and the check (no cap) was marked. The latter contained the typical black stroma with a coating of gray hyphæ.

Warty hyphæ occur quite frequently in the black stroma (observed in cultures 6 days old), but they are not very abundant. Both dark and colorless hyphæ vary greatly in size and frequency of septa even in young cultures. Aerial hyphæ in a culture 7 days old varied from 2 to 8 $\mu$ in diameter. The coarse filaments were many septate, often being divided into isodiametric cells with bulging lateral walls. As the cultures grow older, the hyphæ, both in the surface layer and the stroma, usually become very much distorted and contain many chlamydosporelike swellings. In a culture 5½ months old most of the aerial hyphæ were dark brown, slender, and more or less bristlelike, resembling those in a corn-meal culture of *Lasiodiplodia tubericola* of the same age. There were also some much coarser filaments divided into isodiametric cells with bulging lateral walls. (Pl. VIII, fig. 9.)

*Orange-knot strain on steamed corn meal.*—Comparative cultures were made of the lime strain and of that isolated from the orange knot in January, 1909. The orange strain made a growth which, though resembling that of the lime strain both macroscopically and microscopically, was much more copious.[1] The orange strain, however, produced pycnidia. The first pycnidia discovered were in flask cultures four months old. The cushionlike elevations of the stroma which had appeared during the first four weeks had increased very much in size, some of them having developed into stalklike outgrowths about 4 mm. high and more or less branched Clavarialike. Many of these were honeycombed with chamberlike pycnidia, all of which with one exception were immature. In one case a pycnidium was found which was filled with large, colorless, one-celled, ovoid spores, but, although a great number of sections were cut, not another pycnidium containing spores could be found, nor did all the stalklike outgrowths contain even immature pycnidial chambers. The cultures were examined from time to time during the weeks following, but it was not until two months later that spores were found again. At this date there were many mature pycnidia, and spores had been discharged in cirri. These cirri were 2 to 3 mm. long and yellowish, but later observations of fruiting cultures show that the spore tendrils are usually pure white when first discharged from the pycnidium. Many of the spores in the cirri were septate (one to two septa) and were germinating. Some of them had put forth two germ tubes. By no means were all of the germinating spores septate, however. Occasionally a spore which was not germinating was found with one or two septa. Rarely a two-celled spore occurred which was slightly constricted at the septum. Two-septate spores were sometimes divided into two small terminal cells and a larger central one. The spores measured 20 to 36 μ by 8 to 12 μ. The majority were about 32 by 8 μ.

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[1] The more luxuriant growth of the orange strain may have been due to the fact that it was a more recent isolation.

In addition to the large ovoid spores these 6-months-old cultures contained in the same stalklike outgrowths of the stroma numerous tiny, cylindrical, colorless, continuous, sporelike bodies—so-called spermatia. These were produced either in separate pycnidia or spermagonia buried in the stroma or were present in great numbers in the same pycnidia as the large ovate spores. They were cut off from long slender basidia arranged radially, and measured 4 to 8 μ in length and 1 μ or less in diameter. The interior of the spermagonium was sometimes convoluted so that there were several centers of radiation. The germination of these small sporelike bodies has not been observed.

In 1,000-c.-c. corn-meal flasks inoculated from these 100-c.-c. flask cultures containing spores, the pycnidia developed much more rapidly, i. e., within seven weeks. The little tufts of hyphæ were observed in six days and in eight days looked like little woolly balls and were 1 to 2 mm. in diameter. They grew very rapidly, sometimes doubling in size in 24 hours. Sections of these made the eighteenth, twenty-third, and twenty-ninth days revealed the presence of immature pycnidia, but no spores were found until the forty-eighth day, at which time they were present in abundance. A very few of them were two-celled and occasionally one was germinating. Two days later the spores were being discharged in cirri. In some of the cirri the majority of the spores were two to three celled and were germinating; in others few, if any, had put forth a germ tube, and a septate spore was rarely observed. Plates were poured from the pycnidia, and potato cylinders were inoculated with single spores germinating on these plates. It was one of these potato cultures, originating from a single spore, which was used for the orange-strain inoculations of July 21, 1909 (see p. 54). In the potato cultures mature pycnidia were found in four days, and in a liter corn-meal flask inoculated from the potato culture used for inoculation on July 21, 1909, pycnidia were present and discharging spores within seven days. Thus a vigorously fruiting strain has developed from the orange-knot strain. The pycnidia on corn meal are merely chambers in the stroma and have no distinct pycnidial wall. They are supplied with an ostiole and usually have a short neck which protrudes slightly above the surface of the substratum. The stalklike projections of the stroma were observed only in the small flasks (100-c.-c.) in which there was less moisture and the development was slower. In the liter flasks the pycnidia were produced in any portion of the thin, wrinkled stroma or in cushionlike elevations of it first noticeable as little tufts of hyphæ. The pycnidia were visible to the naked eye as tiny raised points thickly dotting the surface, although they were sometimes more or less concealed by the velvety gray surface growth of aerial hyphæ.

*Prune agar.*—On this medium there is good, but by no means copious, growth. The fungus covers the surface of the slant or plate with a thin layer composed for the most part of horizontal hyphæ. At the advancing edge there occurs in a few days a more dense growth consisting of short, branching, erect hyphæ, frequently in little tufts. In plate colonies that have attained the size of the plate, these hyphæ often form a marginal ring 0.5 to 1.5 cm. in width. They may also be scattered sparingly over the entire surface but are not easily observed with the naked eye.

Plates 9 cm. in diameter containing a single colony (inoculated in the center with a bit of mycelium) were covered by the fungus in 14 to 18 days (temperature 20° to 25° C.). The average growth per 24 hours for the first seven days was seven-eighths of a centimeter and was at a comparatively uniform rate for each colony. The most rapidly growing colony averaged 1 cm., and the one making the least growth 0.75 cm. per 24 hours. Colonies concentrically ringed. (Pl. IX, fig. 3.)

The pure white of very young cultures changes in a few days to grayish white and finally to black, with the exception of the aerial hyphæ, which usually remain grayish white. The dark color has a tendency to appear in concentric rings. Microscopical examination reveals the presence among the horizontal hyphæ of filaments of all gradations of color from hyalin to dark brown, often including some with a decidedly olivaceous tinge, but the macroscopical appearance of an old culture as a whole is black, although in plate cultures the olivaceous tinge is noticeable to the naked eye.

The hyphæ vary greatly in size, amount of branching, frequency of septa, and in general form. In some instances the septa are very infrequent, in others the cells are nearly isodiametric. A large proportion of the branches come off at right angles to the main filament, but they may be at any angle. The cell walls may be straight or undulating, and frequently the hyphæ are twisted imperfectly into loose or closer spirals. These undulating and spirally twisted hyphæ are often very noticeable in old cultures (three to four weeks), although they have been observed in cultures 2 days old. The hyphæ are often very much distorted and chlamydosporelike swellings are of very common occurrence, especially in filaments growing in the agar. The latter may occur in cultures of any age. Swollen hyalin cells may occur in brown hyphæ and vice versa. Sometimes these swollen cells are densely filled with protoplasm, but they do not possess a wall thicker than that of the rest of the filament and in all probability do not function as true chlamydospores.

Early in the growth of a prune-agar culture, sometimes in two days, interwoven hyphæ may be observed. Primary or secondary branches from the same or a neighboring hypha twine about the main filament. On slant agar these little tangles of hyphæ increase rapidly in size until (if they have turned dark in color in the meantime) they are visible to the naked eye as distinct, opaque, black bodies resembling pycnidia. Such bodies have been observed in prune-agar slants 5 days old. These masses of hyphæ may be elongated or irregular in shape but are most commonly approximately round.

Measurements taken from these bodies in a slant 13 days old varied from 40 to 136 $\mu$. In plates the growth of these masses is much less rapid and in plate cultures 4 weeks old the individual hyphæ composing them could easily be distinguished. In a slant 8 months old a cellular tissue had formed very similar to the peridium of a pycnidium or perithecium, but in no case have these bodies been found to contain spores.[1]

The mycelium grows down into the agar but forms no stromatic layer on this medium. In old cultures the agar is so densely invaded by the black hyphæ that the whole is converted into a black mass.

*Beef agar.*—On nutrient peptonized beef agar +15, there is fairly good, though somewhat restricted, growth. Colonies attain the average size of 7 cm. in three to four weeks,[2] while on slant agar the growth is very compact and spreads slowly. On plates there is never a sufficient massing of the hyphæ to render the colony black as a whole, the color never being darker than a deep brown in the darkest portions. Colonies four weeks old were light brown, the darker portions corresponding with more or less uniformity to the concentric rings. There are a few, short (1 to 2 mm.), erect, grayish white, somewhat matted, aerial hyphæ, more abundant near the center of the colony.

Unlike their behavior on prune agar, on tubes of slant beef agar the hyphæ penetrate the substratum little, if any, but form on the surface of the medium a tough, black stromatic layer covered with short, grayish white hyphæ, erect at first, but becoming more or less matted. This layer is formed in about a week and may become one-half of a millimeter in thickness, but extends over only about one-third of the slant, which may or may not be covered by a thin layer of mycelium. In old cultures (4 weeks) the cells of the hyphæ composing the stroma are much swollen and distorted.

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[1] The fruiting orange strains have never been grown on this medium.

[2] On plates containing a single colony.

As on prune agar, the hyphæ vary widely in size, color, and frequency of septa. Coiling filaments, filaments with undulating walls, and fusion of hyphæ are frequent and sometimes in old cultures (4 weeks) the ends of the hyphæ are much swollen.

A striking characteristic of the mycelium grown on this medium is the occurrence, with more or less frequency, of thickenings on the outside of the walls, giving a warty appearance to the filament. These warty hyphæ have been observed in young and old cultures, on plates and slants, and in aerial and stromatic hyphæ. (Pl. IX, fig. 5.)

Interwoven hyalin hyphæ are of frequent occurrence in young cultures, but they increase very little in size and do not develop into black pycnidialike bodies visible to the naked eye, as do the similar knots of hyphæ on prune agar. (See p. 22.) None have been observed too dense to distinguish the individual filaments. (Pl. IX, fig. 6.)

A striking characteristic of the colonies on beef-agar plates was the occurrence of chains of swollen, colorless, chlamydosporelike cells (Pl. IX, fig. 1), particularly abundant near the margin of the colony. The majority of these were thick walled and resembled true chlamydospores. No brown ones were observed except in brown hyphæ. Swollen cells, sometimes full of protoplasm, sometimes nearly empty, are commonly present in all stages in old and young cultures both on slant agar and in plates, but those described resemble true chlamydospores much more closely than any others observed on this medium or on prune agar.

*Potato agar.*—Potato agar affords a most excellent medium for the lime-knot fungus. In one set of seven slant cultures two days after inoculation there was a luxuriant fluffy white growth of erect hyphæ about 2 mm. long, covering an area about 1½ cm. in diameter. Twenty-four hours later the growth had doubled, the erect hyphæ measured 3 to 5 mm., and there was a thin, barely visible layer of horizontal hyphæ extending beyond the mass of erect filaments. In four days the mycelial growth still presented a downy appearance, but was becoming somewhat matted. Five days after inoculation the growth next to the agar in the lower part of the slant was black and had formed a compact, tough stromatic layer. In some tubes this layer was covered with a thick, more or less matted coating of white or grayish white aerial hyphæ, while in others the latter had become a part of the black, compact, stromalike mass, which had a finely roughened surface and contained a few tiny, round, dense masses (visible by transmitted light) resembling the pycnidialike bodies on prune agar. No pycnidia were ever found. The fruiting strains discovered after this work was done have not been grown on slant potato agar.

Sometimes the cultures undergo the changes noted less rapidly, e. g., in two other sets of cultures there was still a thick, fluffy, white, surface growth of erect hyphæ after 10 days, with only a tinge of gray in some of the older hyphæ. Growth sometimes colony-wise.

Growth of the lime-knot strain on +6, +8, and neutral potato agar, Fuller's scale (Pl. VII, fig. 4), varied little, if any, but the orange strain isolated from knots on orange made a much better growth on the +8 agar than on the neutral agar (Pl. VII, fig. 5) and also grew more rapidly on the former than the lime-knot strain.[1] There was no penetration of the agar by the hyphæ, nor was the agar stained.

Colonies on potato-agar plates attained a diameter of 1.5 to 2 cm. in 48 hours.[2] Growth quite dense, but composed of horizontal hyphæ with the exception of a marginal ring of erect hyphæ about 1 mm. long. In two weeks the colonies were 7 to 8 cm. in diameter and still white with the exception of a black ring near the margin. The dark color was first noticeable in 11 or 12 days.

In very young slant cultures many of the hyphæ are rather coarse (4 to 8 $\mu$), have a very few septa, often have undulating walls, and frequently are coiled into rings or spirals. There are also many very fine filaments not exceeding 1 $\mu$ in width. A few days later many of the filaments are considerably distorted and the septa are more frequent.

In very old cultures (8 months) coarse, many-septate filaments (Pl. VIII, fig. 10) and very much distorted hyphæ (both hyalin and colored) are very common. Some of the colored filaments are divided into approximately isodiametric cells with slightly bulging lateral walls. Fused and warty, colored hyphæ are also of common occurrence. (Pl. VIII, figs. 7 and 8.) The color is in the cell wall; hence the warts, being much thicker and therefore blacker than the rest of the wall, give to the hyphæ a mottled appearance.

In young colonies on plates the hyphæ are curling and often so thickly beset with short branches that they have almost a spiny appearance.

In plates poured from spores from the fruiting orange-knot strain, the spores germinated (Pl. VIII, fig. 4) and in many cases produced very long, much-branched hyphæ in less than 24 hours. In five days mature pycnidia were present in abundance. They formed most thickly on the margins of the colonies and made a solid marginal ring. (Pl. IX, fig. 2.) No spermatia were found when the plates were 3 weeks old, but later (41 days) they were present in abundance. At this date some of the large ovoid spores were one to two septate.

*Corn-meal agar.*—Very poor growth on this medium. Surface of the slant barely covered in a week with a thin, almost invisible mycelial layer of horizontal hyphæ. In two cultures 5 weeks old one contained sufficient colored hyphæ to give a decidedly greenish black tinge to the lower half of the slant, while in the second tube barely a trace of color was visible. No interwoven hyphæ were observed and few chlamydosporelike swellings. There was no stromatic layer.

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[1] The more rapid growth of the orange strain was probably due to its more recent isolation.

[2] Each plate contained a single colony.

Colonies in plates of this medium were 6 to 7 cm. in diameter (5 weeks) and had made no growth after the third week.[1] A few aerial hyphæ were in the central portion. About one-half to two-thirds of the hyphæ (5-weeks-old colonies) were colored, practically all the color having appeared after the colonies ceased to increase in size.

Hyphæ rather straight for the most part and very much less distorted than on prune or beef agar. Filaments in plate 5 weeks old measured 2 to 4 $\mu$ in diameter. Found a very few of the warty hyphæ described under growth on beef agar.

*Silica jelly.*[2]—Poor growth. In five days the mycelium covered an area 6 to 8 mm. in diameter. Hyphæ short, rising above the surface scarcely 1 mm., but penetrating the substratum to a depth of 2 to 3 mm. In eight days the area of growth was about 12 to 20 mm. in diameter. Color, dark gray. In 16 days there had been only a very slight increase in growth. The margin of the colony was white or nearly so, while the central portion was nearly black.

In cultures 5 years old there was a tough black stroma about 0.5 mm. thick, covered by a thin brownish gray mycelial layer. No chlamydospores or pycnidia were found.

*Nutrient gelatin slants.*—There is slow liquefaction of gelatin by both the lime and orange strains (fruiting and nonfruiting), the slowness being due in part, no doubt, to the temperature of the ice thermostat in which the cultures were kept. The temperature ranged from 15.5° to 20° C. and averaged 17.5° C., which is far below the optimum for this fungus (see p. 36). Within 24 hours 11 out of 22 cultures showed a sunken area beneath the inoculating material, varying from a slight depression to a pit 2 or 3 mm. deep. There were no signs of growth in any of the cultures. After six days there were still two tubes that showed no signs of liquefaction. In 10 days, however, there was at least a small pit in all the cultures. On the sixteenth day the amount of liquefaction varied from a small pit about 1 mm. deep (lime strain) to about three-fourths of the gelatin (fruiting orange strain). Most of the tubes contained deep pits and considerable liquid gelatin. The fruiting orange strain had led in the amount of liquefaction from the beginning. With the exception of a few tubes the growth on the sixteenth day was restricted to a new outgrowth of short hyphæ on the bit of stroma used for inoculation. In the fruiting orange strain, however, the liquid gelatin was nearly full of fluffy white mycelium and in one of these tubes, on the surface of the slant above the liquid, there was an area of dark-gray growth (0.5 by 1 cm.) of creeping hyphæ on which little cushionlike elevations were forming. In one of the cultures of the nonfruiting orange strain the deep pit which had formed was lined with a good white growth of creeping hyphæ covering an area about 2 by 2.5 cm. in extent.

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[1] Each plate contained a single colony.

[2] Slant cultures.

In 19 days one of the fruiting orange strain cultures had formed a partial dark-gray pellicle composed of hyphæ full of chlamydosporelike swellings.

All of the gelatin in all of the tubes liquefied in 38 days.

*Steamed potato cylinders.*—Excellent growth. The third day (65 hours) there was a thin white mycelial layer covering about one-third the surface of the potato above the water.[1] Twenty-four hours later the whole of the potato above the water was covered with a thick downy white growth of feathery hyphæ 4 to 10 mm. long (24.5° C.). The sixth day the mass of erect hyphæ was decidedly gray, with the exception of the youngest filaments, and was becoming somewhat matted, while next to the surface of the potato a black stroma about one-third of a millimeter thick had formed at the base of the slant next to the walls of the tube. Microscopical examination showed the presence of many swollen cells in the hyphæ both in the stroma and in the surface growth. Individual filaments olivaceous or hyalin, gray or black (in stroma) in mass. Great variation in size of hyphæ. Some coarse brown filaments with nearly isodiametric cells among the erect hyphæ.

When the cultures were 3 weeks old the potato cylinders were practically covered with a thick, wrinkled, black stroma coated by a thin, matted layer of grayish white hyphæ. Two weeks later portions of this external layer were nearly black, while others, a more recent growth, were white (Pl. VII, fig. 2). Small portions of the stroma were white and others were cream buff (Ridgway's Nomenclature of Colors). In one set of cultures, 4 weeks old, of the lime-knot strain, there were cushionlike elevations of the stroma 2 to 5 mm. in diameter. These were full of pycnidialike chambers, but no spores were present. No spores have been found upon potato except in cultures made from the fruiting orange strain.

Potato cultures 3 weeks old were tested with potassium iodid for starch. A microscopical examination was made of the tissues so treated. Aside from a very faint purplish stain in a few cells there was no reaction in any portion of the potato which was covered by the stroma. The tissue of the potato was penetrated by hyalin hyphæ and was much softened. The hyphæ in the tissue were much distorted and full of chlamydosporelike swellings (Pl. IX, fig. 4).

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[1] Growth is sometimes much more rapid than this (Pl. VII, fig. 3).

*Orange strain on potato cylinders.*—The second isolation of the orange strain (isolated July 22, 1909, from a Jamaican knot received in January) behaved on potato in the same manner as the lime strain, making an excellent growth and producing a thick, tough stroma. No spores have been found in the cultures, although this strain has produced pycnidia on the host. (See orange-strain inoculations of July 29, 1909, p. 58.)

The fruiting orange strain, however (see orange strain on corn meal, p. 20), in all cultures that were fruiting abundantly produced instead of a thick, tough stroma a thin, crustlike layer consisting mostly of pycnidia.[1] The pycnidia appeared in great numbers in three or four days, closely crowded together, and were almost concealed at first by the surface hyphæ. In five or six days little round white masses of discharged spores dotted the surface of the culture. Some of the spores in cultures eight days old were pale yellowish, but by far the majority were colorless. In cultures 39 days old spermatia were present in abundance. These were not observed when the cultures were 20 days old.

Sometimes there is little more than the pycnidium wall separating the pycnidia. Sometimes the pycnidium is surrounded by a well-defined subiculum. The fungous layer is thin and easily broken or crushed, in marked contrast to the thick, tough stromatic layer in cultures of the lime strain and the nonfruiting orange strain. The surface of the mycelial layer is thickly dotted with the necks of the pycnidia which protrude slightly above it. Some of the pycnidia have necks nearly as long as the body of the receptacle and are truly flask shaped, while others are nearly spherical and still others spheroidal. These all usually taper, at least slightly, toward the ostiole through which the spores are discharged. Occasionally the wall is so thin that it is transparent and the spores are visible within. In old cultures (41 days) there were many one to two septate spores, although the majority were continuous. One with four septa was observed. Many of the brown septate bodies (see p. 16) were present also (Pl. VIII, fig. 5).

*Steamed banana cylinders.*—Moderate amount of growth in seven days as a thin, delicate, white layer, covering the slanted portion of the cylinders. Banana plainly visible through the fungous growth. Compact matlike growth covering the fluid.

In cultures 5 years old large, brown, thick-walled chlamydospores were present in abundance. These were one to several celled with walls in various planes and were often very irregular in shape (fig. 6). There were also some thick-walled colorless chlamydospores. Stromalike layer about 1 mm. thick covered by a feltlike layer varying in color from light brown or grayish white to black. No pycnidia found.

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[1] If the cultures were fruiting less abundantly the thick, tough stroma was produced, and in this were embedded the pycnidial chambers.

*Steamed apple cylinders.*—Good growth. In cultures 8 days old the cylinders were entirely or partially covered with a rather thin dark-brown, nearly black, mycelial layer. The erect hyphæ extended 4 to 6 mm. above the substratum. There was a decided browning (tawny, Ridgway's Nomenclature of Colors) of the apple which extended beyond the fungous growth. The apple in the check tube was approximately Ridgway's cream buff.

In cultures 9 months old the mycelial layer was about 2 mm. thick, dark brown, and feltlike, and there were small tufts of hyphæ about 2 mm. in diameter in the center of which a small amount of pseudoparenchyma had formed. Microscopical examination was made of cultures at this age. The hyphæ were very dark brown and comparatively straight for the most part, although in some instances the walls were undulating. Filaments varied from 2 to 8 $\mu$ in width, the majority being about 3 $\mu$ wide. Septa frequent. Warty hyphæ present. No swellings observed in the filaments.

Cultures 18 months old and also 5-year-old cultures contained large, brown, one to several celled chlamydospores, resembling those on old banana cultures. Many warty hyphæ and hyphæ divided into approximately isodiametric cells were present in these old cultures. No pycnidia were found.

*Steamed carrot cylinders.*—Steamed carrot is an excellent medium for this fungus. Abundant white growth in four days. Hyphæ rising several millimeters above the substratum giving a woolly appearance to the cultures. In eight days the growth was light gray and tufted. Sixteen days after inoculation the mycelium had become a darker gray and was matted into a feltlike layer in places. No change in the color of the medium.

In cultures 5 years old a few pycnidia were found. There was a rather thin tough stroma covered by a dark-brown, feltlike matted layer of aerial hyphæ. There were a very few large, brown, one to several celled chlamydospores like those described in old banana cultures. In some of the cultures long chains of chlamydosporelike swellings were found in abundance (fig. 7). Some of these were brown and some were colorless, with coarsely granular contents. Usually only cross walls occurred, but occasionally a longitudinal septum was found. Walls thin. Warty hyphæ present. Warts very large, as on lime-wood cylinders. (See p. 31.)

*Steamed macaroni cylinders.*—Good, though rather slow, growth. In four days there was very slight, barely visible growth, hyphæ rising very little from the substratum. In 12 days all of the macaroni above the water was covered by a thin, dark-gray, feltlike mycelial layer. In four weeks the fungous layer had become 1.5 to 3.5 mm. thick, and varied in color from grayish to nearly black. The filaments varied in diameter from 3.5 to 7.5 $\mu$, the finer hyphæ being the more common.

In cultures 5 years old the substratum was covered by a thin, tough, black stroma with a thin, dark-brown, feltlike superficial layer of hyphæ. No pycnidia or chlamydospores were found.

*Steamed orange rind.*—In seven days the fungus had covered the greater part of the surface with a thin layer of mycelium, white for the most part but turning dark in portions. In some of the tubes tiny spherical bodies resembling those described on lime-wood cylinders (see p. 31) were present when the cultures were 10 weeks old.

A very few pycnidia from which the large, hyalin, one-celled spores had been discharged in cirri were found in a culture 17½ months old and also in one 5 years old. No chlamydospores observed. Mycelium grayish to dark brown. Thin, black stroma.

*Steamed parsnip cylinders.*—Copious light-gray growth in eight days, covering all portions of the cylinder above water. Erect hyphæ 4 to 8 mm. long. Small spherical masses of pseudotissue like those described on lime-wood cylinders (see p. 31) in some of the tubes.

A few pycnidia with large, hyalin, one-celled spores discharged in cirri were found in two cultures 5 years old. These cultures also contained a few brown chlamydospores, chains of swollen colorless cells, and a thin, tough, black stroma with a feltlike coating of dark-brown hyphæ.

*Steamed prunes.*—Slight growth in two days, erect hyphæ rising 1 to 2 mm. above the substratum. In seven days there was a thin cobweblike growth of long, branching, colorless hyphæ, 4 to 5 mm. long. Many of these were very coarse (12 $\mu$ in diameter) and were breaking up into one to several celled portions, some of which were germinating. Most of the cells in these portions were approximately isodiametric. There was no bulging of the lateral walls. Sometimes the end walls were rounded, but often they were not.

In cultures 5 years old there was a fairly good growth of brownish gray mycelium. No pycnidia or stroma had formed. Many germinated fragments of colorless hyphæ were present, some with thick walls and some with thin. There also were colored hyphæ breaking up in the same manner, but none were observed which had germinated. Large, brown, thick-walled chlamydospores were present, resembling those in old banana cultures, but smaller for the most part and containing fewer cells.

Cultures of the fruiting orange strain produced a more compact growth of short colorless hyphæ and in seven days were fruiting abundantly. A few septate spores. No long cobwebby hyphæ. No stroma.

In cultures of the nonfruiting orange strain there were in seven days little tufts of short grayish hyphæ dotting the surface of the medium. The interior of these was composed of brown hyphæ but no pseudotissue had formed. In both this and the fruiting orange strain some of the hyphæ had broken into short fragments, but to a very much less degree than in the lime strain.

*Steamed salsify cylinders.*—Good growth on this medium. In cultures 8 days old the portion of the cylinder above the water was covered with a thick feltlike layer of light-gray mycelium. In cultures 5 years old there was a thin, black stromatic layer covered with grayish brown hyphæ. Neither pycnospores nor chlamydospores were observed.

*Steamed sweet potato cylinders.*—Good growth. In seven days all of the cylinder above the water was covered with a thick gray feltlike layer. In cultures 9 months old the mycelium was very dark brown, almost black. Many of the hyphæ were divided into approximately isodiametric cells with bulging lateral walls. In one of a set of five cultures 5 years old, both pycnospores and spermatia were found. The pycnospores measured 16 to 32 $\mu$ by 4 to 6 $\mu$. The spermatia were slightly larger on this medium than on corn meal. They measured 4 to 10 $\mu$ by 1 to 1.5 $\mu$, the majority being 4 to 6 $\mu$. Good, tough, black stroma.

*Steamed tomato.*—Moderate growth in four days, covering the greater portion of the surface of the medium and forming tufts of hyphæ. Mycelium grayish white. Four days later the color was dark gray. In cultures 5 years old there were chains of brown and colorless chlamydospores resembling those on carrot cylinders. No pycnospores found. Thin stromalike layer formed on this medium.

*Steamed turnip cylinders.*—Rather scanty growth in four days. In eight days there was a good gray growth covering the portions of the cylinder above the water and small round masses of pseudotissue had formed. The mycelium in old cultures (9 months) was very dark brown, nearly black. In cultures 5 years old a few cirri of discharged pycnospores were found in each of the six tubes. The cirri in these very old cultures were yellowish. Spores one celled with few exceptions. There were a few warty hyphæ and a very few brown chlamydospores. Thin, tough, black stroma.

*Steamed lime-wood cylinders.*—Some of the tubes contained twice-distilled water, others peptone water, but there was no noticeable difference in the amount of growth made by the fungus on the two media. The cylinders were inoculated with mycelium from cultures 2 weeks old, and in less than 20 hours the growth was sufficient to be seen easily with the naked eye. The hyphæ in some of the tubes were about 1.5 cm. long. In three days most of the cylinder above the water was covered with white hyphæ. The majority of the filaments were creeping, but there was a fairly good, though rather thin, woolly growth of aerial hyphæ.

In five days practically the whole cylinder was covered; mycelium still white. When the cultures were 2 weeks old the hyphæ were more or less matted and were very dark brown for the most part. The wood was colored also (it was found later that the hyphæ had penetrated the cylinders).

Six weeks after inoculation many little spherical, gray bodies were present in the cultures. One of the tubes, with twice-distilled water, contained several hundred of these. (Pl. VII, fig. 1.) They averaged 2 to 3 mm. in diameter and were very hard and attached very firmly to the substratum. Microscopical examination showed them to be composed of a compact mass of pseudoparenchyma, with a covering of short grayish hyphæ. Microtome sections were made of some of these bodies from cultures 2 months old (fig. 8). Some cultures on this medium were kept for five years, but no pycnidia or perithecia ever developed. The mycelium in these old cultures varied in color from a pale brownish gray to a very dark brown and formed a feltlike layer covering the cylinders. No stromatic layer. The hyphæ were much septate and sometimes warty. The warts were much larger than any observed in beef agar, frequently being 2 to 4 $\mu$ thick. In the tubes with peptone water there were varying numbers of chlamydospores. Most of the tubes contained very few, but in one culture there were chains of them, both brown and colorless, most of them one celled. The colorless ones had coarsely granular contents. None were observed in the tubes containing distilled water.

The cylinders were penetrated to a greater or less degree by the hyphæ. In one tube to which sterile water had been added one and two months after inoculation, the hyphæ had penetrated the whole length of the cylinder (5.5 cm.), and the wood and pith were plentifully streaked with black.

*Steamed lemon twigs.*—In three days the portion of the twig above the water was practically covered with a white cobwebby growth. In seven days the mycelium was turning gray and had formed a feltlike layer. Numerous tiny white tufts or cushionlike masses.

In cultures 5 years old the wood was covered with a thin feltlike layer of mycelium varying in color from light brown to dark brown. Cushionlike masses of pseudoparenchyma. No pycnidia or chlamydospores observed.

*Beef bouillon.*—Good, though rather slow, growth in beef bouillon +15 (Fuller's scale). In six days there was an excellent, fluffy, woolly, white growth of hyphæ about 1.5 cm. long. In nine days one of the three tubes had a white pellicle, which 24 hours later had a very faint gray tinge. When the cultures were 4 weeks old, one had a tough black pellicle, another a gray, and the third a white one. In all three the growth in the fluid was pure white and contained many crystals.

In 6-day-old cultures the mycelium was rather fine (the majority of the hyphæ measured 2 to 3 μ), branching, and with few septa. In the cultures 2 months old the septa were much more frequent, the walls were more or less irregular in outline, and there were many incipient branches. The hyphæ in the compact, tough mat forming the pellicle were full of large swellings.

*Raulin's fluid.*—Good growth in this medium. In five days there was a tuft of very white fluffy mycelium about three-fourths of a centimeter in diameter in the bottom of the tube. The growth gradually increased, until at the end of six weeks the fungus filled the liquid and had formed a dark-gray pellicle on the surface. The submerged mycelium was still pure white.

In cultures 5 years old the dark-brown pellicle or stroma had cushionlike elevations 2 to 3 mm. in diameter. Some of these contained pycnidia which were mere cavities in the stroma and from which the spores were discharged in cirri. The spores measured 20 to 32 μ by 4 to 8 μ. Some were quite broad and short (20 by 8 μ), some were long and slender (32 by 4 μ), but 32 by 8 μ was the average size for the majority.

There were a few spermatia and brown chlamydospores present also. The majority of the spermatia were 4 to 5 μ long.

*Fraenkel and Voge's solution.*—There is very poor growth in this medium. Eight days after inoculation the growth consisted of a tuft of grayish mycelium about 0.5 cm. in diameter. When the cultures were 2 weeks old, the tufts had not increased noticeably in size, but they were nearly black. The hyphæ were short, forming a compact mass. The cultures were under observation for six weeks, but there was little if any further growth.

*Milk.*—No change occurred in the appearance of the milk until the fourth day. On that day 8 out of 14 cultures had a translucent band 2 to 10 mm. wide from which the casein was being precipitated. Among these eight tubes were cultures from three strains—the lime and the fruiting and nonfruiting orange strains. The milk below this translucent band was still fluid and opaque, but in some of the tubes it was of a thick creamy consistency. In nearly all of the cultures there was a good, firm, white or gray pellicle. The fifth day soft creamy clots were forming around the submerged hyphæ. The seventh day the band of translucent whey, which was slowly becoming more transparent, was 1 to 2.5 cm. wide in most of the cultures, although in a few tubes precipitation was going on much more slowly.

Two cultures of the nonfruiting orange strain had quite solid clots of casein in the bottom. The cultures of this strain had undergone the changes described more rapidly from the beginning than those of the other two strains. Pycnidia present on the seventh day in the rim and pellicle of the fruiting orange strain. On the eleventh day several of the cultures contained solid clots of casein, filling one-fourth to one-third of the liquid. The whey above this was transparent or semitransparent. In other tubes the casein was being precipitated more slowly and had not made solid clots. In two of the lime-strain cultures the milk was translucent throughout, with the exception of a very small clot in the bottom. In one of the lime-strain cultures the milk above the solid clot, which filled about one-fourth of the liquid, was opaque and between Ridgway's smoke gray and olive gray. Seven days later (cultures 18 days old) the small amount of curd in two of the lime-strain cultures had been dissolved and the fluid was translucent throughout. In the other tubes (all strains) there was still a soft curd, above which the fluid was clear or translucent.

In a later experiment in which the cultures were under observation for four weeks the fluid was clear and there was no curd at the end of that period. There had been a soft curd earlier in the life of the cultures, but this had been completely dissolved.

*Litmus milk.*—When cultures (both lime and orange strains) were made in milk to which sufficient litmus had been added to make it lilac color the translucent portion (see milk cultures, p. 32) became purplish. On the sixth day the color varied in the different cultures from plum purple to Indian purple (Ridgway), while the color of the milk below the translucent band was unchanged. Where there was cream in the rim it was colored rose pink (Ridgway) on this date. This color disappeared later, however. The change in color followed the increase of the translucent or transparent zone, and by the tenth day nearly all of the cultures were prune purple or Indian purple throughout. There had been no indication of any more decided acid production at any time. On the seventeenth day the cultures of the lime strain were all approximately Ridgway's prune purple. Those of the nonfruiting orange strain varied from pansy purple to prune purple and a very deep purple that was nearly black, and the cultures of the fruiting orange strain were prune purple and pansy purple. In one of the cultures of the fruiting strain the lower half of the milk was pansy purple, while the upper half was nearly colorless (slightly brownish yellow) and perfectly transparent except for a few small granules, probably portions of the clot not yet dissolved. All of the cultures in all of the strains were transparent and none contained clots. These cultures were under observation for 35 days. At the end of that time the fruiting orange strain[1] showed complete reduction in one tube and partial reduction in the remaining four tubes. No reduction in the nonfruiting orange or lime strains. Similar results were obtained in another set of experiments in which two tubes of the fruiting orange strain[1] showed partial reduction in 20 days and complete reduction in 38 days,[2] while even at the end of 44 days there was none in the nonfruiting orange or lime strains.

In another experiment, however, one of the nonfruiting orange cultures showed almost complete reduction in 30 days, while no signs of it were exhibited by the other strains. It is probable that all the strains are capable of reducing litmus if sufficient time is devoted to the experiment.

*Association experiment on prune agar (lime strain plus orange strain).*—Prune-agar plates were inoculated on opposite sides with mycelium from corn-meal flask cultures of the lime and orange strain (nonfruiting). No fruiting bodies of any kind appeared but the difference in the amount of growth was most striking. On the twenty-first day the orange strain had made nearly three times as much growth as the lime. Neither strain made any further growth.

## CULTURE MEDIA.

The culture media used in these experiments were prepared as follows:

*Fruit and vegetable cylinders.*—The fruits or vegetables were thoroughly scrubbed with a brush, pared (except prunes and oranges), and washed in distilled water. Cylinders were then cut and put into test tubes with sufficient twice-distilled water to cover them about half way. Tubes either autoclaved for 15 minutes at 110° C. or steamed for 15 to 20 minutes on three consecutive days.

*Lime and lemon wood cylinders.*—Small branches of lime and lemon trees were cut into sections about 2 inches long and put into test tubes (one in each tube), with 10 to 15 c. c. of distilled water. Tubes then autoclaved for 10 minutes at 120° C.

*Macaroni.*—Prepared like wood cylinders.

*Corn meal.*—Corn meal with sufficient twice-distilled water to cover it thoroughly was put into flasks (about three heaping teaspoonfuls of corn meal to a 100-c.-c. flask). Autoclaved 30 minutes at 115° C.

*Potato agar.*—Selected smooth, firm potatoes, washed and scrubbed thoroughly with a brush, pared, and cut into thin slices or shredded, added two parts twice-distilled water, let simmer for half an hour, strained off water, filtered through paper or gauze, and to the filtrate added 1 per cent of agar flour. Steamed 45 minutes, filtered, and tubed. Tubes autoclaved 15 minutes at 110° C.

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[1] One transfer removed from germinating spores in an agar plate.

[2] Seven separate colonies of the fruiting orange strain are represented by these two experiments.

*Prune agar.*—Five dry prunes washed thoroughly under the tap, put into a beaker containing 1 liter of distilled water and allowed to simmer for one hour, then brought to a boil for five minutes. Filtered off juice through filter paper and added 1.5 or 2 per cent of agar flour. Steamed three-quarters of an hour, filtered, and tubed. Titrated usually about +6 Fuller's scale. Tubes autoclaved 15 minutes at 110° C. or steamed for 15 minutes on three consecutive days.

*Apple agar.*—Pared and cut apples into thin slices, added two parts of distilled water. Boiled 20 minutes, strained through gauze and added 1 per cent of agar flour. Steamed one-half hour. Filtered and tubed. Autoclaved 15 minutes at 115° C.

*Apple-twig agar.*—To 500 grams of apple twigs cut into small pieces, added 1,000 c. c. of distilled water. Boiled 20 minutes. Strained through gauze and added 1 per cent of agar flour. Steamed one-half hour, filtered, and tubed. Autoclaved 15 minutes at 115° C.

*Corn-meal agar.*—To 3 liters of distilled water added 12 teaspoonfuls corn meal. Kept in water bath, temperature about 58° C. Strained through gauze, steamed one hour, filtered through paper. Added 1 per cent of agar flour. Steamed one hour, filtered through paper, tubed. Autoclaved for 15 minutes at 115° C.

*Beef agar.*—To 1,000 cubic centimeters of peptonized beef bouillon added 1 per cent of agar flour. Steamed three-quarters of an hour. cooled down to below 60° C., and added the neutralized whites of two eggs to clarify. Steamed three-quarters of an hour, filtered, and tubed. Made +15 Fuller's scale by adding $4\frac{\mathrm{NaOH}}{1}$. Tubes autoclaved 15 minutes at 110° C.

*Silica jelly.*—100 c. c. sodium silicate, sp. gr. 1.15° Beaumé, 100 c. c. HCl 1.10° Beaumé.

Dialized 24 hours in tap water in collodion sacks. Boiled, filtered hot, and cooled in ice water.

To 85 c. c. of above was added 15 c. c. of Fermi's solution. Tubed and autoclaved 15 minutes at 110° C., with vent shut down as soon as steam came out, and kept closed.

*Peptonized beef bouillon.*—Prepared in the manner described in "Bacteria in Relation to Plant Diseases," by Erwin F. Smith (vol. 1, p. 195) and made +15 Fuller's scale.

*Fermi's solution.*—

| | |
|---|---|
| Distilled water | 1,000.0 c. c. |
| Magnesium sulphate | .2 gram. |
| Acid potassium phosphate | 1.0 gram. |
| Ammonium phosphate | 10.0 grams. |
| Glycerin | 45.0 c. c. |

*Fraenkel and Voge's solution.*—To 500 c. c. of redistilled water were added the following:

| | |
|---|---|
| Sodium chlorid | 2.5 grams. |
| Dipotassium phosphate | 1.0 gram. |
| Ammonium lactate | 3.0 grams. |
| Sodium asparaginate | 2.0 grams. |

Steamed 20 minutes, filtered, tubed, and tubes steamed for 15 minutes on each of three consecutive days.

*Raulin's fluid.*—To 1,500 c. c. of redistilled water were added the following:

| | |
|---|---|
| Cane sugar, granulated | 70.00 grams. |
| Tartaric acid, crystals, Merck's | 4.00 grams. |
| Ammonium nitrate C. P., Baker & Adamson | 4.00 grams. |
| Ammonium phosphate, reagent, Merck's | 60.00 grams. |
| Potassium carbonate, standard, E. & A. | 60.00 grams. |
| Magnesium carbonate C. P., Baker & Adamson | 40.00 grams. |
| Ammonium sulphate C. P., E. & A. | 25.00 grams. |
| Zinc sulphate C. P., E. & A. | .07 gram. |
| Ferrous sulphate, crystals, C. P., Merck's | .07 gram. |
| Potassium silicate, dry, E. & A. | .07 gram. |

Steamed 10 minutes at 100° C. Filtered and pipetted into test tubes. Tubes steamed for 15 minutes on each of three consecutive days.

## TEMPERATURE RELATIONS.

Potato cultures of the lime strain of this fungus were placed in thermostats at different temperatures, two tubes placed at each temperature, as shown in Table I. Temperatures were recorded two to three times a day.

TABLE I.—*Thermostat and room temperatures in which potato cultures of lime strain of fungus were kept.*

| Room or thermostat. | Temperatures (° C.). | |
|---|---|---|
| | Average. | Range of variation. |
| Ice thermostat 2 | 3.5 | 2.75 to 4.8 |
| Ice thermostat 10 | 16.8 | 14 to 19 |
| Room temperature | 23.4 | 19 to 27 |
| Thermostat 1 | 31.75 | 30 to 34.5 |
| Thermostat 2 | 35.4 | 33 to 38.5 |
| Thermostat 3 | 44 | 36 to 49 |
| Thermostat 4 | 37.9 | 31 to 43 |

The cultures were under observation for five weeks (daily observation for 2 weeks), but no spores of any kind were formed. No growth took place at 3.5° C. or at 44°, and only scanty growth at 37.9°, while at 16.8°, 23.4°, 31.75°, and 35.4° C. the growth was excellent, though differing widely in rate, as shown in Table II.

TABLE II.—*Reaction of Sphaeropsis tumefaciens on potato to different temperatures.*

| Time of observation. | Average temperature (° C.) and place where cultures were kept. | | | | |
|---|---|---|---|---|---|
| | 16.8° (ice thermostat 10). | 23.4° (room). | 31.75° (thermostat 1). | 35.4° (thermostat 2). | 37.9° (thermostat 4). |
| 18 hours (1st day) | No growth; 14.5° C | No growth; 22.5° C | Growth about 5 mm. in diameter in one tube; 30.25° C. | Small amount of growth in one tube; 33.5° C. | Slight growth in one tube; 40° C. |
| 24 hours (1st day) | No growth; 15.75° C | No growth; 23° C | Good growth in both tubes; long, erect hyphæ; 30.5° C. | Good growth in both tubes, somewhat less than in thermostat 1; 34° C. | Slight growth in one tube; 35° C. |
| 41 hours (2d day) | No growth; 14.75° C | No growth; 23° C | Excellent white growth, nearly covering the surface of the potato above the water; long, white hyphæ; 30.5° C. | Nearly as good growth as in thermostat 1; hyphæ slightly shorter; 33.75° C. | Slightly more growth than yesterday; barely visible, however; 35.25° C. |
| 47 hours (2d day) | No growth; 16.75° C | No growth; 25.5° C | Growing very rapidly; much more growth than 6 hours ago; feathery hyphæ 5 to 6 mm. long; 31° C. | Growing rapidly; long feathery hyphæ. Somewhat less growth than in thermostat 1; 34.75° C. | No change; 37° C. |
| 65 hours (3d day) | No growth; 16° C | Thin white growth covering about one-third the surface of the potato above the water; 24.5° C. | Growth extending down into the water now; slight tinge of gray in small area in one tube; 32.25° C. | No gray tinge; 36° C | Slightly more growth; 41.75° C. |
| 69 hours (3d day) | Very small amount of barely visible growth in tube 5; 16.25° C. | 25° C | 32.5° C | 36° C | No change; 39.5° C. |
| 89 hours (4th day) | Scanty growth in tube 5, covering area about 1 cm. in diameter; small amount of mycelium growing on the walls of tube 6; 17.5° C. | Excellent growth in both tubes; surface of potato above water is covered with a thick downy white growth 4 to 6 mm. high; very great amount of growth in last 24 hours; 24.5° C. | Growth becoming more or less matted. Pale-yellow stromalike layer; 32.25° C. | Resembles growth in thermostat 1; stromalike layer is cream color; 36° C. | Scanty growth covering surface of potato in tube 14; no growth in tube 13; 31° C. |
| 137 hours (6th day) | Half the surface of potato above water is covered with a thin growth of white mycelium in tube 5; tube 6, only half as much growth; 19° C. | Growth excellent and somewhat matted; more abundant growth (i. e., above water) than in thermostat 1; all but the youngest growth is decidedly gray; black stroma next to potato; 25° C. | Growth above water less copious than at room temperature and much less gray, but whole surface of potato, including portion in water, is covered; mycelium in water is white, with exception of yellowish stromalike portion; black stroma above water; 32.75° C. | Very much less growth than in thermostat 1 and at room temperature; surface of potato covered; mycelium white; growth more or less matted; cream color stroma; 36° C. | Very slight growth in tube 13; growth in tube 14 is still only barely visible; 48° C. |

TABLE II.—*Reaction of Sphaeropsis tumefaciens on potato to different temperatures*—Continued.

| Time of observation. | Average temperature (° C.) and place where cultures were kept. | | | | |
|---|---|---|---|---|---|
| | 16.8° (ice thermostat 10). | 23.4° (room). | 31.75° (thermostat 1). | 35.4° (thermostat 2). | 37.9° (thermostat 4). |
| 161 hours (7th day) | Considerably more growth in tube 6, but very scanty in both tubes; 15.75° C. | No change, except that culture as a whole is becoming darker in color; 20.5° C. | Gray color has deepened very much in 24 hours and now extends to the greater part of the growth; much less color than in cultures at room temperature; 31.25° C. | Large gray area in both tubes; no traces of color 18 hours earlier; about as much color as in thermostat 1 cultures 18 hours ago; 34.9° C. | Slightly more growth; 32.5° C. |
| 185 hours (8th day) | Slight increase in growth; 15.5° C. | Little, if any, change; 19° C. | Little, if any, change; 31.25° C. | Little, if any, change; 35° C. | Little, if any, change; 35° C. |
| 214 hours (9th day) | No change; 17.8° C. | Growth extending into the water and becoming darker colored; 24° C. | No change; 31.25° C. | No change; 34.8° C. | Slightly more growth in tube 14; plainly visible now; 43° C. |
| 233 hours (10th day) | Fungus growing very slowly, but amount of growth has doubled in last 48 hours; growth olivaceous in tube 5; 17.9° C. | Little, if any, change; 24° C. | Growth slightly darker in color; 32° C. | No change; 36° C. | No change; 37.5° C. |
| 264 hours (13th day) | Excellent woolly white growth in tube 6; growth in tube 5 is black, but much less in amount; 19° C. | No change | Growth becoming blacker in tube 8; 34.5° C. | Growth in tube 9 becoming darker; 38.25° C. | Good growth in upper half of cylinder in tube 14; hyphæ short; 40° C. |
| 306 hours (15th day) | Black color appearing in tube 6; 16.8° C. | No change; 21° C. | No change; 34° C. | No change; 38.5° C. | No change; 40.5° C. |
| 22d day | Excellent growth in both tubes, though less than at room temperature and in thermostats 1 and 2; grayish white surface growth with tough, black, wrinkled stroma underneath; 15° C. | Excellent growth; cylinders nearly covered with a tough, black, wrinkled stroma; surface hyphæ gray and matted; small portion of stroma is pale buff yellow as in thermostats 1 and 2; more growth than in any of the thermostats; 21° C. | Whole cylinder covered with a tough wrinkled stroma, black for the most part, but with pale yellow portions; 30° C. | Somewhat less growth than in thermostat 1; tough, wrinkled, black stroma, pale yellow in places; 36° C. | Upper third of cylinder in tube 14 is covered with a compact, feltlike yellowish white growth of short matted hyphæ, with a tough whitish stroma underneath; only a very small amount of growth in tube 13 and this has dried out completely; 39° C. |
| 36 days | Cultures resemble those at room temperature very closely, except that coating of grayish white hyphæ over the black stroma is thicker; growth has been excellent, but slow. | Much of the matted layer of surface hyphæ coating the stroma is nearly black, but youngest portions are white; small portions of stroma are a pale yellow or cream buff (Ridgway). | No change | No change | No change. |

On the thirty-sixth day all the cultures were removed to room temperature. Six days later tube 14 from thermostat 4 contained an excellent, gray-white, matted, surface growth with a tough, black, somewhat wrinkled stroma entirely covering the surface of the potato. None of the others had changed in appearance.

No fruiting bodies formed in any of the tubes. The only difference noted in the cultures at the different temperatures was in the rate of growth. On the twenty-second day one of the two tubes growing at each temperature was tested with iodin potassium iodid for starch. All behaved alike. In the portions of the cylinder covered by the stroma there was practically no starch reaction, i. e., there was only the faintest purplish stain in a few cells, so slight as to have been easily overlooked. The tissue of the potato was much softened and was penetrated by hyphæ (Pl. IX, fig. 4). The microscopical characters resembled those of cultures grown at room temperature (described under growth on steamed potato cylinders, p. 26).

## INOCULATION EXPERIMENTS.

Inoculations have been made on lime, orange, pomelo, lemon, kumquat, tangerine, and *Citrus trifoliata.* These have been numbered in two series, one comprising those made with the lime strain, the other with the strain isolated from the orange. A detailed account of the experiments follows.

### INOCULATIONS (LIME STRAIN) ON LIME, DECEMBER 1, 1904.

The first inoculations were made on three healthy lime trees obtained from Florida; grown for some time in a bed in one of the Department greenhouses. The trees were about 3 to 4 feet tall and were making a good growth. The cultures used were: (1) Culture 6 weeks old on sterile lime wood (Pl. VII, fig. 1); and (2) beef-bouillon culture 2 months old. In both cases the fungus had been growing on artificial media 2 months (isolated October 1, 1904). The inoculations were made as follows:

No. 1, Tree I, on young growing wood 4 inches from the end of a growing branch. A small T-shaped cut was made in the bark with a sterile knife and mycelium inserted. The bark was smoothed down again and the stem wound with raffia, which completely covered the wound.

No. 2, Tree I, on new green wood just below a fork, one branch of which was 7 inches long, the other (on which was inoculation No. 1) 12 inches in length. Inoculation made as in No. 1.

No. 3, Tree I, on an older branch with grayish bark, 2 inches from the trunk. Diameter of inoculated branch, three-fourths of an inch. Inoculated like No. 1.

No. 4, Tree I, on a stem similar to No. 3, but somewhat smaller. Inoculated like No. 1.

No. 5, Tree II, on a rather old stem with gray bark, three-fourths of an inch in diameter. Inoculated like No. 1.

No. 6, Tree III, on a younger branch with green bark. Inoculation made by pricking the stem three times with a sterile needle, then placing a little mycelium over these punctures and binding with raffia.

Nos. 1 to 6 were inoculated from the lime-wood culture.

No. 7, Tree II, on a low side branch 8 inches long. Inoculation midway on stem, made like No. 1.

No. 8, Tree I, on the trunk about 3 feet from the ground. Mycelium bound with raffia on an old wound where a branch had been broken off.

No. 9, Tree III, on a branch with gray bark. Inoculated near the trunk by removing a small piece of the outer bark and binding mycelium over the cut surface.

No. 10, Tree II, on a gray stem near the trunk. Inoculation made by cutting off a very small amount (2 to 3 sq. mm.) of the outer bark, then spreading the mycelium over this injury and binding it with raffia.

Nos. 7 to 10 were inoculated from the beef-bouillon culture.

A year after the inoculation (December 1, 1905) characteristic knots had formed in every case except No. 8, in which no new wound was made. Some of these knots were then 1½ inches in diameter and resembled in every particular those received from Jamaica. No. 4 was photographed (Pl. IV, fig. 2) and pure cultures of the fungus were obtained from it.

From both Nos. 4 and 7 a witches'-broom had been produced. In No. 4 there had been an extensive growth of the main stem beyond the knot, but in No. 7 there had been none.

Tree I was photographed on March 31, 1906 (Pl. III).

On April 14, 1906, one year and four and one-half months after inoculation, the following detailed observations were made:

No. 1. Knot about 1¼ inches in diameter. The surface is rather rough and somewhat cracked, and the color is quite a dark brown or almost black in places. There are about 4 feet of growth beyond this knot, all of which is dead. This is the first knot which has caused the death of that portion of the limb beyond it.

No. 2. This knot is on the trunk of the tree and is somewhat larger and lighter in color than No. 1. It has not caused the death of the wood beyond it.

No. 3. Knot on the trunk about 1¾ inches in diameter. It is somewhat cracked and lighter in color than the bark on the adjoining stem. About half a dozen shoots are growing out of it, all of which are apparently healthy.

No. 4. Cut out on December 1, 1905.

No. 5. Knot about 1 inch in diameter, badly cracked and dark in color. A witches'-broom has formed and one of the shoots is about 4 feet long.

No. 6. Knot about 1 inch in diameter and quite irregular in shape; surface smooth and lighter colored than the surrounding bark; witches'-broom from this knot also.

No. 7. Removed December 1, 1905.

No. 8. No knot. This is the one inoculated on the old healed wound.

No. 9. Knot 1½ inches in diameter; surface smooth and light colored. One of the several shoots which has grown from this knot is about 8 feet long and as thick as a man's thumb at the base.

No. 10. Knot about an inch in diameter, very rough and scarred. A portion of the surface is almost black, while the remainder is very light colored. There are several shoots growing from this knot, one of them being over 3 feet in length. None of the growth above the knot shows any signs of disease.

On November 17, 1906, nearly two years after inoculation, all parts of the trees above the knots, with the exception of No. 5, were dead or showed signs of disease. Inoculation No. 9 on a branch at the base of one of the largest trees (Tree III) at this time was a knot nearly the size of a man's fist. Gum was oozing from it in numerous places and one of the branches above this inoculation, bearing another knot (inoculation No. 6) had been dead for many weeks. The remaining parts of this tree were still green but were making little or no growth. The leaves were very flabby and turning yellow. They were beginning to curl and dropped very easily. A large branch below the knot was very green and growing rapidly, a striking contrast to the branches above the point of inoculation, where the new growth, if any, was not at all vigorous, the young leaves being almost devoid of chlorophyll.

This tree (Tree III) at this time furnished a most interesting example of the effect of inoculation with the lime-knot fungus. Inoculation No. 6, on one of the smaller limbs, had caused the death of the branches above it some months before. The trunk of the tree, which bore a very large knot near the base, the result of inoculation No. 9, was dying, while one large branch springing from a point below the knot was apparently as healthy as ever.

Nos. 5 and 10, on Tree II, had grown into large knots and the branches above No. 10 were all dead. No. 5, which was on one of the branches of the fork of the trunk of Tree II, was a much smaller knot than No. 9, described above. The parts of the tree above No. 5 were still green and apparently healthy.

On Tree I all of the branches above inoculations Nos. 1 and 2 were dead; the rest were green and healthy.

On September 17, 1907, nearly two years and ten months after inoculation, the trees were still alive and were then growing rapidly. The portions above the inoculations were dead in most cases, but many branches had grown out below these centers of infection. Many secondary knots had appeared on one of the trees (Tree II). These extended from the ground, where they occurred in the greatest numbers, all the way up the trunk, and they varied in size from half an inch to 2 inches in diameter. Young shoots had grown from many of the knots.

In July, 1908, it became necessary to remove the lime trees from the bed in which they had been growing and, believing that the experiment had continued long enough, they were cut out. The condition of the one showing the greatest number of knots (Tree II) was as follows:

The main stem had a diameter at the surface of the earth of 2½ inches and a diameter at the height of 1 foot above the surface of 1½ inches. This diameter was preserved upward for about 40 inches from the earth. About 3 feet from the ground on a branch of the fork of the main stem was inoculation No. 5, and on a smaller branch given off just below it was No. 10, these tumors being dried out and black. At the fork the inoculated branch was three-fourths of an inch in diameter, while the uninoculated one had a diameter of 1¼ inches.

The most striking thing in connection with the stem was the great number of secondary tumors occurring throughout a distance of about 3 feet. The surface of the stem was almost entirely covered with them. There were 100 or more. The exact number can not be stated, because of fusions. A few of these secondary tumors were dead, but nearly all were living and covered by a grayish white bark, occasionally somewhat fissured. The length of these secondary tumors varied somewhat, being from three-fourths of an inch to 1½ inches; their breadth from half an inch to 1 inch or more; and their height from one-fourth to three-fourths of an inch. Most of them were elliptical in outline, the longest axis being in the direction of the long axis of the stem. These swellings, as already stated, were attached by a wide base firmly to the wood of the stem. Some of these secondary tumors were sending out feeble shoots, half a dozen to a dozen or more in places. One vigorous shoot from the base of the stem had a basal diameter of three-fourths of an inch and a length of 5 feet or more. There were some secondary swellings at the base of the stem and two tumors of moderate size about 1½ feet above the base. It was at first thought that these might be the result of more recent natural infections because of the distance between the tumors, but microscopical examination revealed the presence of the fungus in the wood in small quantities throughout the intervening space. Six inches farther up the main stem another vigorous shoot had attained a length of 4 feet or more and bore two small secondary tumors near its base. The general appearance of the trunk of this tree is well shown in the photographs, which, however, were made from the shrunken, dried-out stem (Pls. V and VI).

Gum was oozing from the stem in places, but not more than one would expect in cases of wounds due to other causes.

A year later, July 22, 1909, the fungus was isolated from one of the secondary knots on the trunk of this tree (Tree II), which was cut down the previous year and has been in the dry laboratory ever since. Thus the mycelium has lived in these knots at least 2 years—1 year dry. Inoculations made with this isolation on tender shoots of lime and pomelo on July 29, 1909 (see p. 57), produced within 3 weeks unmistakable tumor tissue and mature pycnidia and spermagonia.

*Summary.*—All of the inoculations with the exception of No. 8, in which no wound was made, have been most successful. The observations extended over a period of 3½ years. In the majority of cases the portion of the tree above the knot was dead and many witches'-brooms had formed both from the artificial infections and the secondary knots. The fungus was traced through the stem for a distance of 5 feet beyond the point of inoculation (Pl. V, x to x′).

### INOCULATIONS (LIME STRAIN) ON LIME AND ORANGE, DECEMBER 12, 1905.

On this date 10 more inoculations were made, 5 (Nos. 11 to 15) on lime and 5 (Nos. 16 to 20) on orange. None of the trees had ever been inoculated, and all were free from knots. Inoculations were made by making a T-shaped cut in the bark, inserting mycelium, and binding the wound with raffia. A bouillon culture of the fungus made on December 1, 1905, directly from one of the artificial infections in the greenhouse,[1] was used for the inoculations. The orange trees had been recently transplanted and were in a dormant state. There were no young, growing shoots on them.

On April 14, 1906, four months after inoculation, all the inoculations on the limes were taking, as follows:

No. 11. Slight but distinct swelling at the point of inoculation.

No. 12. Swelling about the size of a split pea and lighter colored than the rest of the stem. The limb beyond the knot was somewhat stunted.

No. 13. Slight but distinct swelling at the point of inoculation. Diseased area lighter colored than the surrounding bark.

---

[1] No. 4, December 1, 1904.

No. 14. Knot about half an inch in diameter, light in color. Very little growth has been made beyond the point of inoculation.

No. 15. Slight swelling for about half an inch along one side of the stem. No definite knot, although there was slightly more swelling at the exact point of inoculation.

There were no signs of disease on the orange trees (varieties not recorded), and with the exception of No. 16, where there was very slight swelling, it was most difficult to determine the point of inoculation.

Seven months later there were still no signs of infection on the oranges.

On September 17, 1907, No. 11 (lime) bore a small knot the size of a kernel of corn. In No. 12 there was a small rough knot at the point of inoculation and a slight swelling extending along the branch for a short distance.

November 13, 1908.

Limes. Trees not growing well.

Nos. 11 and 12. Tumors have increased little, if any, in size during the past year.

No. 13. Tumor tissue, but no true knot.

No. 14. Missing. Had a knot about half an inch in diameter four months after inoculation.

No. 15. Missing. Tumor tissue four months after inoculation.

*Summary.*—Limes. Small knots formed in three of the five lime inoculations and tumor tissue in the other two.

Oranges. No infection, with the possible exception of No. 16, in which there was slight swelling. The trees were not in good condition when inoculated.

### INOCULATIONS (LIME STRAIN) ON POMELO, LEMON, CITRUS TRIFOLIATA, KUMQUAT, AND LIME, JULY 5, 1906.

Inoculations were made on pomelo, lemon, *Citrus trifoliata,* kumquat, and lime. Pure beef-bouillon cultures made directly from a small artificially produced knot were used and inoculations were made on twigs and leaves. In the former the method used in the earlier experiments was employed.

Nos. 21 to 25 (pomelo, Duncan variety). The trees were small and had been set out but a short time, so were not growing rapidly. No. 25 was inoculated on a leaf. The midrib was pricked and mycelium was placed over the wound.

Nos. 26 to 30 (lemon, Villa Franca variety). These trees were also small and making little growth. No. 30 was made on a leaf in a manner similar to No. 25.

Nos. 31 to 35 (on a seedling lemon 8 to 10 inches high). It was very healthy and growing rapidly. Nos. 33 and 35 were made on leaves.

Nos. 36 to 40 (*Citrus trifoliata*, two trees). No. 40 was on a leaf, the other four on the stem.

Nos. 41 to 45 (Japanese kumquat). All inoculations on twigs.

Nos. 46 to 48, 51, and 52 (old lime trees). Nos. 46 to 48 were made on leaves, the others on twigs.

On November 7, 1906, the following observations were made on these inoculations:

Nos. 21 and 22 (pomelo). Slight swelling at the point of inoculation.

No. 23 (pomelo). Stem considerably swollen for about half an inch.

No. 24 (pomelo). The branch in which this inoculation was made has grown 1½ feet during the summer. Point of inoculation could not be found.

No. 25 (pomelo leaf). No evidence of infection.

Nos. 26 to 35 (lemon). No signs of disease.

No. 36 (*Citrus trifoliata*). Slight swelling at the point of inoculation.

No. 37 (*Citrus trifoliata*). Slight swelling about one-half inch long.

No. 38 (*Citrus trifoliata*). Small knot about the size of a small pea. The effect produced here by inoculation is, at this date, greater than on the limes inoculated at the same time.

No. 39 (*Citrus trifoliata*). Slight swelling at point of inoculation.

No. 40. No record.

The *Citrus trifoliata* plants, as a whole, are not very healthy, but one or two branches are making good growth.

No. 41 (kumquat). Slight scar, no swelling.

No. 42 (kumquat). Very little swelling at point of inoculation.

No. 43 (kumquat). Like No. 41.

No. 44 (kumquat). Very little, if any, swelling.

No. 45 (kumquat). No scar. Point of inoculation not distinguishable.

Nos. 46 to 48 (lime leaves). No signs of infection.

Nos. 51 and 52 (lime twigs). Slight swelling at point of inoculation.

On September 17, 1907, one year and two months after inoculation, the following observations were made:

Nos. 21 and 22 (pomelo). Slight swelling.

No. 23 (pomelo). A large irregular scar about 1 inch long and half an inch wide. This is considerably swollen. The fungus has apparently made some headway here, although it has failed to produce a knot.

No. 24 (pomelo). No infection.

Nos. 31 to 35 (lemon). No results from inoculation. In most cases the point of inoculation is not distinguishable.

No. 36 (*Citrus trifoliata*). Very slight swelling at point of inoculation.

No. 37 (*Citrus trifoliata*). Little, if any, swelling.

No. 38 (*Citrus trifoliata*). Small knot about the size of a pea and similar to the smaller knots on the lime trees. This has grown very little in the last 10 months. The surface of the knot is light in color, rough and cracked, and resembles in every way that of the knots on lime.

No. 39 (*Citrus trifoliata*). Very little swelling.

Nos. 41 to 45 (kumquat). No evidence of infection.

Nos. 51 and 52 (lime twigs). No record.

November 13, 1908. Nos. 21 and 22 (pomelo). Slight swelling.

No. 23 (pomelo). Considerable swelling, but no real knot.

No. 24 (pomelo). Could not find point of inoculation.

Nos. 26 to 29 (lemon). No infection. No scar in Nos. 26, 27, and 29.

Nos. 31 to 35 (lemon). Place of inoculation not discoverable.

No. 36 (*Citrus trifoliata*). Knot about three-fourths of an inch long, one-fourth of an inch high, and three-eights of an inch wide, extending over half way around the stem. It is light colored with a fairly smooth surface and resembles the smaller knots on lime. It is the largest knot which has been produced on *Citrus trifoliata*.

No. 37 (*Citrus trifoliata*). Not a real knot, but there has been much more than an ordinary wound reaction. Swelling one-eighth of an inch high. (Nos. 36 to 39 are on the same plant.)

Nos. 41 to 45 (kumquat). Inoculation places not found.

No. 51 (lime). No knot, only slight swelling.

No. 52 (lime). Label has fallen off, so could not be sure of the point of inoculation. There is a slight swelling on one of the branches, but no true knot anywhere on the tree. Either none has been produced or it has been cut off by the gardener.

November 10, 1909. On this date the trees were removed to another greenhouse, and the following notes were made by Dr. Smith:

> No. 23 (pomelo). Well-defined small tumor at the point of inoculation. This is about 5 mm. in diameter, and as high. Two cm. above and below are similar small secondary tumors.
>
> No. 37 (*Citrus trifoliata*). Some small tumors in the scar tissue.
>
> No. 38 (*Citrus trifoliata*). Slight but distinct tumor in the scar tissue. It is raised up above the level of the stem about 4 mm. and has a diameter either way of about 6 mm.
>
> July 20, 1910. No. 37 (*Citrus trifoliata*). Small tumors and much tumor tissue in the wound. Swollen area is 1.5 cm. long by 5 mm. wide by 3 mm. high.
>
> No. 38 (*Citrus trifoliata*). Small knot 6 mm. in diameter.
>
> No. 39 (*Citrus trifoliata*). Swelling 6 mm. by 6 mm. by 1 mm. high.

*Summary.*—The result of this set of inoculations may be summed up as follows:

TABLE III.—*Results of inoculations of July 5, 1906.*

| Kind of tree. | Knot. | Swelling. | Failure. |
|---|---|---|---|
| Pomelo | 1 | 2 | 1 |
| Lemon | 0 | 0 | 7 |
| Citrus trifoliata | 3 | 1 | 0 |
| Kumquat | 0 | 0 | 5 |
| Lime | 0 | 2 | 0 |

### INOCULATIONS (LIME STRAIN) ON LEMON, POMELO, CITRUS TRIFOLIATA, AND LIME, JUNE 23, 1908.

Inoculations were made with the fungus isolated March 11, 1908, from a secondary tumor on one of the trees first inoculated in the greenhouse. A slit was made in the bark with a small sharp knife and bits of mycelium from a corn-meal culture 8 days old were inserted into the wounds, which were then bound with raffia. The inoculations were numbered as follows:

Nos. 53 to 58, lemon, Villa Franca variety.
Nos. 59 to 63, pomelo, Duncan variety.
Nos. 64 to 67, pomelo, Duncan variety (on another tree).
Nos. 68 to 71, lemon, Villa Franca variety.
Nos. 72 and 73, pomelo, Duncan variety.
Nos. 74 to 76, lemon, Villa Franca variety
Nos. 77 to 82, *Citrus trifoliata.*
Nos. 83 to 88, lime.

Most of the inoculations were made on the younger growth, but some were on the older part of the branch. No entirely sound lime tree was available for inoculation, but the young growth of the one least affected was used for this purpose. No tender, rapidly growing shoots on the trees.

August 4, 1908. Knot beginning to form on No. 61 (pomelo); cut off and put into alcohol.

October 14, 1908. The lemons show little or no results from the inoculation. On some of the pomelos, however (Nos. 59, 62, 63, 65, and 72), knots appear to be forming. The inoculations on *Citrus trifoliata* have also caused some swelling, but it is less than that on the pomelos. The limes inoculated as checks were destroyed at the time of the removal of the trees from one greenhouse to another.

October 15, 1908. Nos. 66 and 81 cut off and put into alcohol. Both show slight swelling.

November 13, 1908. The inoculated trees appeared as follows:

Lemon:

No. 54. Very little swelling, probably wound reaction only.

No. 55. Slight swelling; appears to be more than mere wound reaction.

No. 56. Like 55.

No. 57. Like 54.

No. 58. Slight swelling.

Nos. 68 to 70. Little or no swelling.

No. 71. Some swelling. It may be only wound reaction.

Nos. 74 to 76. Very little swelling, probably only wound reaction.

Pomelo:

No. 59. Slight swelling, but looks like more than a wound reaction.

No. 60. Swelling 8 mm. long and about 4 mm. high. Knot seems to be forming.

No. 61. Cut off August 4.

No. 62. Swelling about 1½ cm. long and about 2 mm. high.

No. 63. Swelling extending about half way around the branch. It is about 2 mm. high.

No. 64. Considerable swelling, about 8 mm. long.

No. 65. Swelling about 1½ mm. long.

No. 66. Cut off October 15, 1908.

No. 67. Slight swelling. (Made on the trunk.)

No. 72. Small knot about 4 mm. in diameter.

No. 73. Little, if any, swelling.

Nearly all of the pomelos look as though knots were forming.

*Citrus trifoliata:*

Nos. 77 to 79. Considerable swelling. Knots probably are forming.

No. 80. Slight swelling.

No. 81. Cut off October 15, 1908.

No. 82. Slight swelling.

Nos. 77 to 79 are on one tree; Nos. 80 to 82 on another.

December 2, 1908. End of pomelo branch on which is inoculation No. 60 is dead. A small knot about 1 mm. long and 2 cm. high. Cut off.

February 11, 1909. No change.

August 20, 1909 (14 months).

Lemon. In no case has a knot formed. On some of the inoculated branches there has been a slight swelling, but it is little, if any, more than a wound reaction. Immature pycnidia are present on Nos. 68 and 70.

*Citrus trifoliata.* Two fairly good small knots have formed on the *Citrus trifoliata* trees. One (No. 77) measures 5 by 3 by 2 mm. and another (No. 79) measures 8 by 4 by 2 mm., ca. In No. 77 a second small swelling 3 mm., ca., in diameter, is present. The rest of the *Citrus trifoliata* inoculations show more or less swelling, and in one case (No. 80) there are a few mature pycnidia.

Pomelo. There has been little, if any, increase in the amount of swelling since November, 1908, at which time considerable tumor tissue had formed. Immature pycnidia formed on the bark of No. 64.

A year later, November 10, 1909, the following observations were made by Dr. Smith prior to the removal of the trees to another greenhouse:

No. 62 (pomelo). Scar bears in its upper portion a well-defined small tumor. This is a roundish one about 5 mm. in diameter and about 4 mm. high.

No. 63 (pomelo). Swollen at the point of inoculation but no well-defined tumor. About 2 inches above, at the origin of the leaf, there is a small nodosity covered with buds.

No. 65 (pomelo). The whole punctured area, which is about 2 cm. long, has developed a stunted tumor, which has not grown any recently. (Inoculation on a terminal shoot which has made no growth.)

No. 73 (pomelo). At the extreme upper part of this scar there is a well-developed small tumor about 4 mm. in diameter. (Inoculation is at the base of a branch half an inch in diameter.)

No. 77 (*Citrus trifoliata*). This inoculation, 3 feet from the end of a small shoot, bears two distinct tumors in the scar. They are rounded up, a gray white, and as plain tumors as any we have ever obtained, although yet small. The largest one is about 6 by 5 by 4 mm. The other is about half the size.

No. 78 (*Citrus trifoliata*). Small shoot with slight evidence of tumor tissue in the scar.

July 20, 1910. No. 77 (*Citrus trifoliata*). Two good, though small knots, one 6 by 6 by 4 mm., the other nearly as large. These are on the lips of the wound.

No. 78 (*Citrus trifoliata*). Tumor tissue.

No. 79 (*Citrus trifoliata*). Knot 9 by 7 by 3 mm.

August 14, 1910. No. 77 (*Citrus trifoliata*). Surface covered with pycnidia, containing the large, hyalin, 1-celled spores. Cut off and taken into the laboratory.

*Summary.*—Pomelo. Small but distinct tumors formed at 6 of the 11 points of inoculation and more or less tumor tissue at 4 others. One was doubtful.

*Citrus trifoliata.* Good, though small, knots in two of the six inoculations and tumor tissue in the other four. Pycnidia.

Lemon. No knots and little, if any, swelling.

Lime. Lost or destroyed during the removal from one hothouse to another early in the course of the experiment.

### INOCULATIONS (LIME STRAIN) ON LIME, JUNE 27, 1908.

Another set of inoculations was made on limes. These were young trees (Tahiti and Florida seedling) which had just been received from the nursery and they had been cut back severely for transplanting. All the young twigs had been removed, so that all the inoculations were made on the trunks. The method employed was the same as in the preceding experiments.

Nos. 89 to 94 were inoculated from corn-meal flask culture 2, June 15, 1908.

Nos. 95 to 100 were inoculated from corn-meal flask culture 3, June 15, 1908.

Both cultures were descended from an isolation made March 11 from one of the secondary tumors occurring on one of the trees first inoculated.

November 13, 1908. With the exception of the two trees on which are inoculations Nos. 95 and 100, all have died back below the point of inoculation. On these two trees, however, knots appear to be forming.

No. 95. Swollen place about 1½ cm. long and 2 mm. high. This is still partially covered by the old bark, which has become lighter in color.

No. 100. Shows only slight swelling, but knot seems to be forming.

August 20, 1909. There has been no increase in the amount of tumor tissue within the last nine months.

*Summary.*—Tumor tissue formed in two cases. In all the others the trees which had been pruned for transplanting died back to a point below the place of inoculation.

### INOCULATIONS (LIME STRAIN) ON LEMON, TANGERINE, AND KUMQUAT, JULY, 1908.

Inoculations were made by the gardener under Dr. Smith's direction. Pieces of bark, including a bit of the wood from young lime knots, were grafted into some trees in the orange house.

December 2, 1908. The following notes were made by Dr. Smith:

> Lemon (American Wonder variety). In each of the three inoculations on lemon (all on the same tree) small tumors have formed in the scar tissues. The largest is now 8 mm. long, 4 mm. wide, and 4 mm. high. These have doubled in size in the last six weeks.
>
> Tangerine (St. Michael). One of the two inoculations looks hopeful.
>
> Kumquat. No swelling at points of inoculation.

February 11, 1909.

Lemon. The tumors in the scar tissues have increased very much in size. The largest is about 2 by 1½ by 1 cm. high. Gum is oozing from some of them.

Tangerine. Knots forming very slowly, if at all. One of the inoculations looks hopeful.

Kumquat. Very little swelling.

June 16, 1909. Photographed one of the knots on lemon (Pl. X). They have increased comparatively little in size in the last few months. This tree was a very vigorous lemon only a few years old and therefore was supposed to be in good condition for infection.

August 20, 1909.

Tangerine. In one of the wounds a knot 5 mm. in diameter has formed. The edges of the cut are considerably swollen in both inoculations.

Kumquat. Considerable swelling in the wound in one inoculation. No reaction at all in the others.

November 10, 1909. Dr. Smith made the following notes on the lemon prior to its removal to another greenhouse:

> Considering the length of time which has elapsed, the development of the knots on this variety of lemon has been very slow. The conditions at present are as follows: Tree full of fruit, which is of very large size. The lowest point of inoculation is about 3 feet from the ground in stem now nearly 2 inches in diameter. The scar bears irregular tumor tissue not much elevated; also a central, well-developed, rounded-up tumor which is about 2 cm. by 2 cm. by 15 mm. high. About one-fifth of a foot up the stem is another scar, which bears eight small tumors varying in size from 1 to 1.2 cm. in diameter. The third wound, on another branch, bears two small tumors on one side of the scar. These are each about 1 cm. in diameter.

January 27, 1911. The knots on the lemon have increased in size astonishingly of late, considering the slow rate at which they have developed hitherto. The largest knot is now about 2.5 cm. in diameter.

*Summary.*—Lemon. Excellent tumor formation in every case.

Tangerine. Small tumor in one case and tumor tissue in the other.

Kumquat. Small amount of swelling in one instance; otherwise no result.

### INOCULATIONS (ORANGE STRAIN) ON LIME, FEBRUARY 24, 1909.

Twelve inoculations were made on lime trees with the fungus isolated January 14, 1909, from orange knots received from Jamaica. A potato-agar slant culture 28 days old was used for the purpose, and the inoculations were made in the way described under the lime-knot-fungus inoculations. Nos. 3 and 10 were made on the trunk and the others on the youngest green twigs available. The inoculations were made on trees which had been growing in the greenhouses for about eight months. There were no tender, rapidly growing shoots.

On April 15 there was noticeable swelling at the point of inoculation, and four weeks later (May 13) signs of infection were unmistakable in the following:

No. 1. Knot about 1 cm. long and 0.75 cm. thick.

No. 2. Much less swelling than in No. 1, but knot appeared to be forming.

No. 4. Stem at point of inoculation swollen to about twice the diameter of the rest of the branch.

No. 5. Slight swelling, but indicative of knot formation.

Five months after inoculation (July 29) inoculation No. 1 was removed. It had increased very little in size since May, but in the mean time the branch bearing it had died.

Microscopical examination of the knot showed the black mycelium present in abundance, especially in the inner layer of bark.

On August 13, 1909, all the inoculations with the exception of the two on the trunk (Nos. 3 and 10) and one other (No. 12) showed signs of infection, as follows:

No. 2. Knot about 1.25 cm. long and 0.75 cm. thick.

No. 4. Good small knot about 1 cm. long and 0.5 cm. thick.

No. 5. Considerable swelling.

No. 6. Considerable swelling.

No. 7. Slight swelling.

Nos. 8 and 9. Considerable swelling, knots clearly forming.

No. 11. Slight swelling.

There was at this date a striking difference between this set of inoculations and those made with the lime-knot fungus 24 hours later (Feb. 25, 1909, see p. 53). In the latter there was only very slight swelling at the point of inoculation. Potato-agar slant cultures 4 weeks old were used in both cases. The orange strain used in this set of inoculations, however, had been isolated only six weeks, whereas the lime strain used for the inoculations of February 25 had been growing for 11½ months on artificial media.

On November 10, 1909, the following notes were made by Dr. Smith prior to the removal of the trees to another hothouse:

> No. 2. Two well-developed small tumors, each about 1 cm. in diameter. The inoculated branch is small and stunted (about 4 mm. in diameter).
>
> No. 4. The scar bears a distinct tumor about 1.5 cm. by 8 mm. by 4 mm. high.
>
> No. 5. A distinct tumor in the scar. It is about 2 cm. long and not much raised above the surface of the stem.
>
> No. 6. Stunted small shoot bearing a distinct small tumor in the inoculated part.
>
> No. 7. The scar has welled-up edges, suggestive of tumor tissue.
>
> No. 8. A stunted small shoot. Distinct tumor tissue in the scar.
>
> No. 9. Shoot stunted. Tumor tissue in the scar.
>
> No. 11. Distinct small tumor at the base of the shoot.
>
> No. 12. The extreme tip of the shoot has not grown any. No tumor.

On July 20, 1910, the trees were just beginning to recover from the shock of transplanting and were beginning to put out new shoots. The following observations were made on that date:

No. 4. Little, if any, growth of the tumor since November, but that part of the inoculated branch above the knot (20 cm.) is dead. Mature pycnidia are present on the surface of the knot, and microscopical examination revealed the mycelium extending 12 cm. above the tumor.

No. 5. Little, if any, change since November.

No. 6. Tumor tissue 8 cm. long by 4 mm. wide by 3 mm. high, making the inoculated portion of the stem twice the size of the rest of the branch.

No. 7. Knot 8 by 6 by 4 mm. There is also in the wound a second knot about half the size, with considerable swelling of the stem between the two.

No. 8. The inoculated portion of the stem is swollen and there are two knots in the wound. One is 0.5 cm. in diameter and the other about 7 by 4 by 2 mm.

No. 9. Considerable tumor tissue in the wound.

No. 11. Knot 8 by 8 by 4 mm.

*Summary.*—Typical tumor formation in 9 of the 12 inoculations. The inoculated shoots were stunted and in one instance all of that portion beyond the knot (20 cm.) died. The black mycelium was traced through it for 12 cm. beyond the knot. Two of the inoculations which failed to cause infection were on the trunk and the third was on a shoot which made no growth after the inoculation. Mature pycnidia were observed on one tumor only (No. 4).

### INOCULATIONS (LIME STRAIN) ON LIME, FEBRUARY 25, 1909.

On this date 12 inoculations (Nos. 101 to 112) were made on small lime trees which had been growing in the greenhouse since June, 1908. Three trees were inoculated. Two of the inoculations were on the trunk, the rest on fairly young green shoots, and were made in the manner previously described. No tender, rapidly growing shoots on the trees. Mycelium from a potato-agar slant 29 days old was used (descended from an isolation of March 11, 1908).

August 13, 1909. There was very slight swelling in all except the two trunk inoculations. Knot seems to be forming slowly in No. 105.

November, 1909. Trees transplanted. No record.

July 7, 1910. No knots have formed. The whole branch bearing inoculations Nos. 102 and 103 is dead.

*Summary.*—No formation of tumor tissue. The trees were small and stunted at the time of inoculation, and some months later several of the branches (uninoculated as well as inoculated) were dying.

The failure of the inoculations may be due to this condition of the trees or to loss of virulence of the fungus, this strain having grown 11½ months on artificial media.

### INOCULATIONS (LIME STRAIN) ON LIME, JULY 21, 1909.

Three very young tender shoots on one of the old lime trees were inoculated. This tree had been previously inoculated, but with little success. It had not been growing well previously, but it was at this time putting forth a few vigorous shoots. Potato-agar culture 7 weeks old was used (descended from isolation of March 11, 1908).

August 13, 1909. No. 113. Stem swollen at point of inoculation to one and one-half times the diameter of the rest of the shoot.

No. 114. Considerable swelling; shoot not growing well. Cut out and examined microscopically. Mycelium found in the tissues.

No. 115. Little, if any, swelling.

The shoots grew very rapidly for a few days after inoculation, but are now growing slowly, if at all.

July 7, 1910. No. 113. Considerable swelling.

No. 115. Very little swelling.

*Summary.*—More or less swelling, but no knots were produced. This is the same strain used for the lime inoculations of February 25, 1909. It has now been growing on artificial media for 14⅔ months and it is quite probable that it has lost its virulence.

### INOCULATIONS WITH PYCNOSPORES (ORANGE STRAIN) ON LIME AND POMELO, JULY 21, 1909.

The inoculations were made from a potato culture (originating from a single spore) 6 days old, the surface of which was thickly dotted with round white masses of the large, colorless, one-celled spores discharged from the pycnidia buried in the stroma. This strain was isolated six months prior to the date of these inoculations. A slit was made in the bark with a sterile knife and a quantity of mycelium and spores inserted. Wound bound with raffia. The inoculations were numbered as follows:

Lime:

Nos. 13 and 14, on same tree, bearing only fairly young branches. No tender shoots.

Nos. 15 to 17, on second tree, bearing only fairly young branches. No tender shoots.

Nos. 18 to 20, on very tender shoots on a third tree.

Pomelo:

Nos. 21 and 22, on young branches of pomelo previously inoculated with lime-knot fungus with little or no effect. These branches or shoots were young, with leaves still pale green, but had already become woody.

All of these trees had been inoculated before, but no large knots or even fair-sized ones had been produced and no secondary knots ever formed. There were very few tender shoots on the trees, but some were beginning to push out. Eight days after inoculation the shoots were growing rapidly and gum was exuding from the wounds.

On August 13, 1909, three weeks after inoculation, the fungus was fruiting on three of the stems (Nos. 15 to 17). There was a slight swelling of the woody tissues, and the black mycelium had grown all through the strip of bark cut for the insertion of the inoculating material and had produced pycnidia filled with the characteristic large, hyalin, 1-celled spores. Some of the pycnidia were buried deeply in the bark tissues; others were bursting through the epidermis. There was no stroma and little or no subiculum. The epidermis had turned grayish white. The detailed observations of the inoculations are as follows:

Nos. 13 and 14. Slight swelling. Not very vigorous shoots.

No. 15. Slight swelling. Small amount of gummy exudate. Pycnidia breaking through the epidermis.

No. 16. Slight swelling. No gum. Pycnidia breaking through the epidermis.

No. 17. Resembles No. 16, but there is less swelling. No gum. Pycnidia breaking through to the surface.

No. 18. Broken off some days ago. There was slight swelling.

No. 19. Some swelling. No gummy exudate.

No. 20. Considerable swelling. Large amount of gum.

Nos. 21 and 22 (pomelo). Little, if any, swelling.

Measurements were made of the pycnidia and pycnospores from inoculation No. 15. The former measured 152 to 192 $\mu$ by 152 to 160 $\mu$, the latter 20 to 32 $\mu$ by 8 $\mu$.

August 20, 1909.

Lime.

Nos. 13 and 14. No change.

No. 15. Edge of the wound much swollen. Mature pycnidia.

No. 16. Good production of tumor tissue. Mature pycnidia.

No. 17. Considerable tumor tissue. Mature pycnidia.

No. 19. Considerable tumor tissue.

No. 20. Stem at point of inoculation is twice its normal size.

Pomelo.

No. 21. Considerable tumor tissue. Mature pycnidia bursting through the epidermis, which has become grayish white. The tissue beneath it is so full of the black mycelium that it appears black.

No. 22. Little, if any, swelling. Tissues appear black.

Gum is no longer exuding and has not been produced in any inoculations additional to those noted on August 13.

November 10, 1909. Notes made by Dr. Smith prior to removal of trees to another hothouse:

No. 13. Twig growing very slowly. Definite small tumor, but it is not growing very rapidly. It is 1 cm. by 3 mm. by 3 mm. high and covers the whole wound.

No. 14. Upper half of the inoculated place bears a distinct small tumor about 5 by 4 by 3 mm. Twig stunted. Scarcely any growth since inoculation.

No. 15. This inoculation has made marked progress since August 13. There is no longer a particle of doubt that the spores found in cultures of the orange strain belong to the life cycle of this fungus. The tumor is cork yellow in color, 1.5 cm. long and 1 cm. wide in the widest portion, and nearly surrounding the stem, which is not over 2 to 3 mm. in diameter.

No. 17. Decided growth in the last 1½ months. Tumor is now a greenish white, cracking open, and nearly 1 cm. in diameter. The stem is about 3 to 4 mm. in diameter. There is a similar tumor on the other side of the stem a little lower down, i. e., at the extreme base of the point inoculated.

No. 20. There has been evidence of a small tumor. Shoot has not made much growth.

July 7, 1910. The trees are just beginning to recover from the removal to the new greenhouse and are putting forth new shoots.

Lime.

No. 13. Inoculated shoot dead. It has made little, if any, growth since inoculation. The dead tip, which is 6.5 cm. long, is covered with pycnidia full of the large hyalin continuous spores. The tumor has increased little, if any, in size since November, 1909.

No. 14. Inoculated tip (6.5 cm. long) is dead and covered with pycnidia. The tumor has increased little, if any, in size in the last 8 months.

No. 15. Good knot 1.5 cm. by 1 cm. by 7 mm. Swelling extends about 2 cm. beyond the knot (covering the whole wound). Small second knot also in the wound. Portion of branch beyond the knot is dead. Pycnidia buried in the wood.

No. 20. All of inoculated branch beyond the knot (5 cm. in length) is dead. Many pycnidia on the stem below the knot. The latter has increased little, if any, in size in the last eight months.

December 17, 1910.

No. 15 now bears a knot about 2 cm. in diameter.

March 9, 1911.

No. 15. Knot 4.5 cm. in diameter girdling the stem.

No. 17. End of branch dead. Removed.

*Summary.*—Limes. Knots were produced on all (5) that were under observation for more than a month, and tumor tissue in the three others; pycnidia appeared on six, and in five cases that portion of the branch beyond the knot died.

Pomelo. These were under observation only a month. During that period pycnidia and considerable tumor tissue formed on one, while the other showed little, if any, sign of infection.

### INOCULATIONS (LIME STRAIN) ON LIME, KUMQUAT, AND POMELO, JULY 29, 1909.

On this date another set of inoculations was made on tender shoots of lime, kumquat, and pomelo. Mycelium from potato culture seven days old (isolation of July 22, 1909, from secondary knot on Tree II, inoculated December 1, 1904) was inserted into the stem in the manner previously described.

Inoculations were numbered as follows:

Lime.

Nos. 116 and 117, very young, tender shoots, not at all woody.

Kumquat.

Nos. 118 and 119, young, very slightly woody.

Pomelo.

Nos. 120 and 121, young rapidly growing shoots. One slightly woody at point of inoculation, the other not at all hard.

August 13, 1910. Slight swelling in both lime inoculations. Little, if any, in the kumquat and pomelo.

August 20, 1909. Mature pycnidia and spermagonia pushing through the epidermis in No. 116. There has been a copious growth of mycelium under the epidermis, but no stroma has been formed. There is slight but unmistakable swelling.

Both of the pomelos show a very good production of tumor tissue, considering the short time which has elapsed since the inoculation (22 days). There has been as much swelling as in inoculations of the same date made with the orange strain. In No. 120 the swelling measures 1.5 cm. by 4. mm. by 2 mm., ca., and in No. 121 it is 1 cm. by 4 mm. by 2 mm., ca.

Little, if any, swelling in the kumquats.

July 7, 1910.

No. 116. Label lost during removal to new hothouse.

No. 117 (lime). Distinct tumor tissue.

No. 120 (pomelo). Swelling 1.5 cm. by 1.5 cm. by 0.25 cm., extending half around the stem.

No. 121 (pomelo). Tumor 1.75 cm. by 0.5 cm. by 4. mm., ca. The whole tip of the branch (7.5 cm. beyond the knot and 1.25 cm. below) is dead, and thickly covered with the pycnidia of this fungus.

*Summary.*—Lime. Tumor tissue formed in both of the inoculated shoots, and pycnidia and spermagonia on one of them (three weeks).

Pomelo. Typical small tumors in both cases. One of the inoculated branches died and was covered with the pycnidia of *Sphaeropsis tumefaciens.* A Diplodia, closely resembling *Diplodia natalensis* Pole Evans and producing a rot of lemons and apples similar to that caused by the latter, was also found fruiting on this dead branch and isolated from it. It is plainly distinguishable from the Sphaeropsis, however, even when immature.

Kumquat. Little, if any, results from the inoculation. Under observation only 22 days, the labels being lost on removal to the new hothouse.

### INOCULATIONS (NONFRUITING ORANGE STRAIN) ON LIME, KUMQUAT, AND POMELO, JULY 29, 1909.

Six inoculations (Nos. 23 to 28) were made on tender, rapidly growing shoots on trees in the greenhouse. Pieces of the stroma from a potato culture 7 days old (a second isolation, July 22, 1909, from the Jamaican orange knots, containing no spores) were inserted into slits in the bark and the wound was bound with raffia. The inoculations were numbered as follows:

Lime.

Nos. 23 and 24, shoots very tender and not at all woody.

Kumquat.

Nos. 25 and 26, young shoots but slightly woody.

Pomelo.

Nos. 27 and 28, very tender rapidly growing shoots. Leaves not entirely unfolded.

August 13, 1909.

No. 23 (lime). Considerable swelling. Small amount of gum.

No. 24 (lime). Slight swelling. Gummy exudate. Cut off and examined microscopically. Mature pycnidia bursting through the epidermis. Pycnidia measured 176 to 192 $\mu$ by 120 to 122 $\mu$. Spores 16 to 24 $\mu$ by 6 to 8 $\mu$.

Nos. 25 and 26 (kumquat). Little, if any, swelling.

Nos. 27 and 28 (pomelo). Slight swelling. Small amount of gum.

August 20, 1909.

No. 23 (lime). Pycnidia bursting through the epidermis in great numbers. Abundant growth of mycelium beneath the epidermis, but no stroma. Swelling about 2 mm. thick.

No. 27 (pomelo). Swelling 5 by 4 by 2 mm., ca.

No. 28 (pomelo). Swelling 1 cm. by 3 mm. by 2 mm. Gum is no longer exuding from the wounds.

Nos. 25 and 26 (kumquat). Little, if any, swelling.

August 31, 1909.

No. 23 (lime) brought into the laboratory for sections. Considerable tumor tissue.

November 10, 1909. Notes made by Dr. Smith prior to the removal of trees to another greenhouse:

> No. 27 (pomelo). On a stunted terminal shoot which has not grown much since the date of inoculation there is a distinct small tumor following the whole of the scar. This is about 1 cm. long by 8 mm. broad by 3 mm. high.
>
> No. 28 (pomelo). A small stunted branch. Overgrowth of the edges of the wound to such an extent that I think it is abnormal but not so well defined a tumor as the preceding.

July 7, 1910.

No. 27 (pomelo). Swelling 1 cm. long by 3 mm. high and extending half way around the stem.

No. 28 (pomelo). This tumor has grown little in the last 8 months. It is now about 1 cm. long by 3 mm. high and extends half around the stem.

*Summary.*—Lime. Tumor tissue and great numbers of pycnidia in both cases. Under observation only 22 to 30 days.

Pomelo. Small tumors in both cases. Inoculated shoots stunted.

Kumquat. Little, if any, result from the inoculation. Under observation only 22 days, the labels being lost on removal to new hothouse.

### INOCULATIONS WITH PYCNOSPORES (ORANGE STRAIN) ON LIME, JULY 20, 1910.

Six inoculations were made on a lime tree which had been growing in the hothouse since June, 1908. It was putting forth many vigorous young shoots at the time of inoculation. A potato culture 9 days old which was fruiting abundantly was used (descended from isolation of January 14, 1909). The inoculations were numbered as follows:

No. 29. Vigorous young tender shoot 20 cm. long (i. e., new growth), inoculated 12 cm. from tip.

No. 30. Tender vigorous shoot 13.5 cm. long, inoculated 4.5 cm. from base.

No. 31. Vigorous tender shoot 16 cm. long, inoculated 6 cm. from base.

No. 32. Shoot young but woody, 18 cm. long, inoculated 8 cm. from base.

No. 33. Like No. 32, 21.5 cm. long, inoculated at base of shoot.

No. 34. Branch 5 mm. in diameter, inoculated at base.

Spores and mycelium were inserted into slits made in the bark or epidermis with a sterile knife. Wounds bound with raffia. Checks made also.

August 2, 1910. Inoculated shoots are vigorous and growing well.

No. 29. Considerable tumor tissue. Shoot has grown 9.6 cm. since inoculation. Mature pycnidia present.

No. 30. Broken off on July 23.

No. 31. Considerable tumor tissue. Shoot has grown 16 cm. since inoculation. Mature pycnidia present.

No. 32. Pycnidia.

No. 33. Very slight swelling.

August 17, 1910.

No. 32. Very little swelling but many mature pycnidia and a browned area extending laterally 2 mm. beyond the wound. The shoot has made no growth since inoculation. Many of the brown septate bodies (see description of fungus) are present.

No. 33. Considerable tumor tissue and many pycnidia. The shoot has not grown since inoculation.

No. 34. Slight swelling at point of inoculation.

September 6, 1910. Knots on all except No. 32, which shows only a comparatively small amount of tumor tissue.

December 17, 1910.

No. 29. Good knot 1.5 cm. long by 1 cm. wide. No growth of shoot since August 2.

No. 31. Fine knot encircling the branch and about 1.75 cm. in diameter. Surface light colored and somewhat fissured. No normal bark. No pycnidia observed at this date (present on the bark August 2). The shoot has made no growth since August 2.

No. 32. Knot 1 cm. long by 0.5 cm. wide.

No. 33. Knot about the size of No. 32.

No. 34. Excellent knot about 1.3+ cm. long by 1.3+ cm. wide. Some of the original bark remains, but it is very light colored. No pycnidia observed.

All have formed excellent knots. The check shows nothing. None of the inoculated shoots have grown since August 2. The normal bark is gone for the most part and no pycnidia were observed to-day.

January 14, 1911. Brought No. 32 into the laboratory and made cultures from it for the reisolation of the fungus. Mycelium not present in great abundance.

January 25, 1911. The strain isolated from No. 32 eleven days ago has proved to be a vigorously fruiting one.

March 9, 1911.

No. 29. Branch dying 9 cm. below and 4.5 cm. above the knot.

No. 31. Knot 3 cm. in diameter.

No. 33. Whole branch dead. Cut off.

No. 34. Knot 3 cm. by 1.5 cm. by 0.75 cm.

June 20, 1911.

No. 29. Of inoculated branch 1½ feet are dead.

No. 31. Inoculated branch is dead.

No. 34. Inoculated branch is losing its leaves and two small shoots branching from it about 3 inches above the knot are dead.

*Summary.*—Excellent knots on all with the exception of No. 30, which was broken off three days after inoculation. The young tender shoots which were growing well at the time of inoculation continued to elongate rapidly for at least two weeks. The more woody ones did not increase in length and had probably ceased to grow prior to the time of inoculation. Pycnidia present on three shoots within two weeks after inoculation and on a fourth two weeks later. Three of the infected branches are dead and the fourth is dying (11 months). The strain of *Sphaeropsis tumefaciens* used for these inoculations was isolated 1½ years before, but had been fruiting abundantly throughout the whole series of cultures in which it had been growing since that time. The tree was making an unusually good growth at the time of inoculation.

### INOCULATIONS (LIME STRAIN) ON LIME, JULY 20, 1910.

Three inoculations were made to-day on a lime tree which had been growing in the hothouse since June, 1908. A potato culture nine days old was used.

No. 124. Young but woody branch, 2 mm. in diameter, inoculated 20 cm. from the tip.

No. 125. Young woody branch 2 mm. in diameter, inoculated at a point 12 cm. from the tip and at the base of a tiny shoot 1 cm. long.

No. 126. Woody branch 4 mm. in diameter.

Incision made in the bark with a sterile knife, and mycelium inserted. Wound bound with raffia.

July 23, 1910. No swelling.

August 2, 1910. Shoots making good growth.

August 12, 1910. Shoots vigorous and growing well. No swelling.

September 6, 1910. No tumor tissue has been produced.

December 17, 1910. No more reaction than shown by the checks.

*Summary.*—No formation of tumor tissue. The strain of the fungus used for these inoculations had been growing 2⅓ years on artificial media and had never been known to fruit. It had probably lost its virulence.

## GENERAL REMARKS ON INOCULATIONS.

Successful inoculations have been made on lime, pomelo, lemon, tangerine, and *Citrus trifoliata.* Only one trial (five inoculations) was made on oranges, the trees not being available. As knots occur on oranges in the field and as infection took place in the limes inoculated the same day with the same culture, the failure of the experiment was probably due to the fact that the trees were not in good condition or were not of a susceptible variety.

The pedigrees of the cultures used for inoculation have been traced and a comparison of these indicates that a gradual loss of virulence accompanies long-continued cultivation on artificial media, unless the strain is a fruiting one. (See Table IV.) Some of the best results (see lime inoculations (orange strain) of July 20, 1910, p. 59) were obtained with a strain which had been isolated 1½ years, but which had been fruiting vigorously in the various transfers made throughout that period. On the other hand only a comparatively small amount of swelling was produced on tender rapidly growing shoots inoculated with a nonfruiting strain which had been isolated 14⅔ months (see lime inoculations (lime strain) of July 21, 1909, p. 54), and inoculations of vigorous young shoots with a young culture of the same strain 2⅓ years after isolation failed altogether (see lime inoculations (lime strain) of July 20, 1910, p. 61). This strain had been virulent 3½ months after isolation. (See pomelo inoculations of June 23, 1908, p. 47.) Woody tissues are apparently as susceptible to infection as young, rapidly growing ones.

Table IV summarizes the results of the inoculations and various facts of interest regarding the cultures used in making them.

TABLE IV.—*Summary of results of inoculations, classified by host plants.*

LIME INOCULATIONS MADE THROUGH WOUNDS ON TRUNKS OR BRANCHES.

| Date and strain. | Culture used for inoculation. | | | | Time under observation. | No. of inoculations. | Percentage of— | | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Age. | Medium. | Descended from— | Time since isolation. | | | Knots. | Swellings.[2] | Failures. | |
| Dec. 1, 1904, lime. | *a*, 6 wks<br>*b*, 2 mos | Sterile lime-wood cylinder.<br>Beef bouillon | *b*<br>Jamaican knot directly. | 2 mos<br>...do | 3½ yrs | 9 | 100 | 0 | 0 | Trees growing well at time of inoculation; inoculated branches killed in most cases; many witches'-brooms formed. |
| Dec. 12, 1905, lime. | 11 days | ...do | Artificial infection; No. 4, Dec. 1, 1904, directly. | 11 days | 3 yrs | 5 | 60 | 40 | 0 | Trees had been recently transplanted and were in a dormant state when inoculated. They did not make a very good growth after inoculation. |
| July 5, 1906, lime. | (?) | ...do | Artificial infection directly. | (?) | 2⅓ yrs | 2 | 0 | [3] 100 | 0 | |
| June 27, 1908, lime. | 12 days | Steamed corn meal | Isolation No. 4, Mar. 11, 1908. | 3½ mos | 1⅙ yrs | 12 | 0 | 17 | 83 | Trees had been cut back severely and transplanted just before inoculation and all except two (83 per cent) died back to a point below the point of inoculation soon after the experiment began. |
| Feb. 24, 1909, orange. | 4 wks | Potato agar | Isolation No. 2, Jan. 14, 1909. | 6 wks | 1½ yrs | 12 | 75 | 0 | 25 | Two of the failures were inoculations on the trunk. All other inoculations were on young though woody shoots. Inoculated shoots stunted and in one case killed. Pycnidia observed on one tumor. |
| Feb. 25, 1909, lime. | ...do | ...do | Isolation No. 4, Mar. 11, 1908. | 11½ mos. | 1½ yrs | 12 | 0 | 0 | 100 | Inoculations were made on small stunted trees. They were making little or no growth and some months later several of the branches were dying. |
| July 21, 1909, lime. | 7 wks | ...do | ...do | 14⅔ mos. | 1 yr | 3 | 0 | 100 | 0 | Three very young tender shoots were inoculated. These shoots grew very rapidly for a few days after inoculation; then grew very little, if at all. |

[1] Inoculations made on unwounded bark were unsuccessful, and inoculated leaves fell off before results could be recorded.
[2] Tumor tissue produced, but not in sufficient quantity to be termed a knot.
[3] Slight.

TABLE IV.—*Summary of results of inoculations, classified by host plants*—Continued.

LIME INOCULATIONS MADE THROUGH WOUNDS ON TRUNKS OR BRANCHES—Continued.

| Date and strain. | Culture used for inoculation. | | | | Time under observation. | No. of inoculations. | Percentage of— | | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Age. | Medium. | Descended from— | Time since isolation. | | | Knots. | Swellings. | Failures. | |
| July 21, 1909, orange, spores. | 6 days[1] | Steamed potato | Isolation No. 4, Jan. 14, 1909. | 6 mos. | 23 days to 1 yr. 5 mos. | 8 | 63 | 37 | 0 | Five fairly young and three very young tender shoots were inoculated. Four of the former formed knots and the fifth was removed at the end of 23 days for sections. Of the three very tender shoots one formed a knot, one was broken off soon after inoculation, and the third was under observation only a month (label lost at time of removal to new greenhouse). Shoots continued to grow rapidly for some days after inoculation. Knots produced on all which were under observation for more than a month. Pycnidia appeared on six of the inoculated shoots. In five cases the portion of the branch beyond the knot died. |
| July 29, 1909, lime. | 7 days | do | Artificial infection directly. | 7 days | 22 days to 1 yr. | 2 | 0 | 100 | 0 | Very young tender shoots were inoculated. One was under observation only 22 days (label lost on removal to new hothouse); but slight swelling was shown in 15 days; mature pycnidia and spermagonia on one of the inoculations in 22 days. |
| July 29, 1909, orange. | do | do | Natural infection directly. | do | 22 to 30 days | 2 | 0 | 100 | 0 | Very tender shoots were inoculated. One was cut off for sections in 1 month; the other was under observation only 22 days (label lost on removal to new hothouse). Pycnidia appeared in great numbers on one in two weeks; on the other in three weeks. |
| July 20, 1910, orange, spores. | 9 days[1] | do | Isolation No. 4 Jan. 14, 1909. | 1½ yrs. | 11 mos. | [2] 5 | 100 | 0 | 0 | Tree was very thrifty, making an unusually good growth when inoculated. Two of the inoculations were made on very young tender shoots which continued to elongate rapidly for at least two weeks thereafter; two were made on young but woody shoots; a fifth was made on a branch 5 mm. in diameter. The woody shoots and the branch had probably ceased to grow prior to the time of inoculation. Pycnidia appeared on four within two and four weeks. A vigorously fruit- |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | ing strain was isolated from one of the knots. Three infected branches were dead and another was dying in 11 months. |
| July 20, 1910, lime. | ...do..... | .....do.................. | Isolation No. 4, Mar. 11, 1908. | 2⅓ yrs... | 5 mos......... | 3 | 0 | 0 | 100 | The inoculated shoots were 2 to 4 mm. in diameter and continued to be vigorous and to grow well after the inoculation, probably owing to loss of virulence of the fungus. |

POMELO INOCULATIONS MADE THROUGH WOUNDS ON BRANCHES.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| July 5, 1906, lime. | (?)...... | Beef bouillon.......... | Artificial infection directly. | (?)...... | 2⅓ to 3⅓ yrs.... | 4 | 25 | 50 | 25 | Trees were not growing well at time of inoculation; had been set out but a short time. |
| June 23, 1908, lime. | 8 days... | Steamed corn meal.... | Isolation No. 4, Mar. 11, 1908. | 3½ mos.. | 1⅓ yrs......... | 11 | 55 | 36 | 9 | Trees were not growing well when inoculated. |
| July 21, 1909, orange, spores. | 6 days[1].. | Steamed potato....... | Isolation No. 4, Jan. 14, 1909. | 6 mos... | 1 mo.......... | 2 | 0 | 50 | 50 | Young, but rather woody, shoots were inoculated. Shoots grew rapidly for a few days after inoculation; under observation only one month (labels lost during removal to new hothouse). Pycnidia were produced on one of the inoculated shoots. |
| July 29, 1909, lime. | 7 days... | .....do.................. | Artificial infection directly. | 7 days... | 1 yr........... | 2 | 100 | 0 | 0 | Young, rapidly growing shoots were inoculated. In one case the inoculated branch was killed and covered with pycnidia. |
| July 29, 1909, orange. | ...do..... | .....do.................. | Natural infection directly. | ...do..... | .....do......... | 2 | 100 | 0 | 0 | Very tender, rapidly growing shoots were inoculated; infected shoots stunted. |

LEMON INOCULATIONS MADE THROUGH WOUNDS ON TRUNK OR BRANCHES.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| July 5, 1906, lime. | (?)...... | Beef bouillon.......... | Artificial infection directly. | (?)...... | 2⅓ yrs......... | 7 | 0 | 0 | 100 | Four of the inoculations were on small trees making little growth when inoculated; three were on a seedling lemon 8 to 10 inches high, which was in very good condition and growing rapidly. |
| June 23, 1908, lime. | 8 days... | Steamed corn meal.... | Isolation No. 4, Mar. 11, 1908. | 3½ mos.. | 14 mos........ | 13 | 0 | 0 | 100 | Trees were not growing well when inoculated. |
| July, 1908, lime. | | Bits of young lime knot grafted into wounds. | | | 2½ yrs......... | 3 | 100 | 0 | 0 | A very vigorous tree a few years old. American Wonder variety. |

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[1] Fruiting abundantly.

[2] A sixth inoculation was made, but the branch was broken off accidentally three days later.

TABLE IV.—*Summary of results of inoculations, classified by host plants*—Continued.

| Date and strain. | Culture used for inoculation. | | | | Time under observation. | No. of inoculations. | Percentage of— | | | Remarks. |
|---|---|---|---|---|---|---|---|---|---|---|
| | Age. | Medium. | Descended from— | Time since isolation. | | | Knots. | Swellings. | Failures. | |
| CITRUS TRIFOLIATA INOCULATIONS MADE THROUGH WOUNDS ON BRANCHES. | | | | | | | | | | |
| July 5, 1906, lime. | (?) | Beef bouillon | Artificial infection directly. | (?) | 2⅓ to 4 yrs. | 4 | 75 | 25 | 0 | |
| June 23, 1908, lime. | 8 days | Steamed corn meal | Isolation No. 4, Mar. 11, 1908. | 3½ mos. | 3½ mos. to 2 yrs. | 6 | 33⅓ | 66⅔ | 0 | Trees were making poor growth at time of inoculation. Pycnidia were observed on two of the inoculated shoots. |
| ORANGE INOCULATIONS MADE THROUGH WOUNDS ON BRANCHES. | | | | | | | | | | |
| Dec. 12, 1905, lime. | 11 days | Beef bouillon | Artificial infection No. 4, Dec. 1, 1904, directly. | 11 days | 11 mos. | 5 | 0 | [1] 20 | 80 | Trees had just been transplanted and were in a dormant state when inoculated. |
| TANGERINE INOCULATIONS MADE THROUGH WOUNDS ON BRANCHES. | | | | | | | | | | |
| July, 1908, lime. | | Bits of young lime knot grafted into wounds. | | | 1 yr. | 2 | 50 | 50 | 0 | |
| KUMQUAT INOCULATIONS MADE THROUGH WOUNDS ON TRUNK OR BRANCHES. | | | | | | | | | | |
| July 5, 1906, lime. | (?) | Beef bouillon | Artificial infection directly. | (?) | 2⅓ yrs. | 5 | 0 | 0 | 100 | |
| July, 1908, lime. | | Bits of young lime knot grafted into wounds. | | | 1 yr. | 3 | 0 | 33⅓ | 66⅔ | |
| July 29, 1909, lime. | 7 days | Steamed potato | Artificial infection directly. | 7 days | 22 days | 2 | 0 | 0 | 100 | Young, slightly woody, shoots were inoculated; under observation only 22 days (labels lost on removal to new hothouse). |
| July 29, 1909, orange. | do | do | Natural infection directly. | do | do | 2 | 0 | 0 | 100 | Do. |

---

[1] Very slight.

## INOCULATIONS ON RAW FRUITS (LIME AND ORANGE STRAINS).

*Inoculations on lemons.*—Ripe lemons which had been soaked 15 to 20 minutes in $HgCl_2$ (1 to 1,000) and then rinsed in distilled water were inoculated by inserting bits of mycelium or spores under the skin. Both lime and orange strains of the fungus were used. The lemons were then placed under a dry bell jar. In three days the inoculated area was soft and slightly browned. The diseased area spread rapidly until the fruit was softened throughout and the entire surface discolored. The rind became black ultimately and was coated with grayish white mycelium. Within a month the lemon inoculated with spores of the fruiting orange strain was covered with a thin stroma filled with pycnidia discharging whitish to yellowish masses of spores, many of which were germinating. The majority of the spores were continuous and colorless, but many were yellowish and there were also numbers of one to five celled spores. (Pl. VIII, fig. 2.) A few days later a few of the brown septate bodies previously described (p. 16) were observed.

Repetitions of the experiment gave similar results. Checks under the same bell jar remained sound.

For comparison, inoculations were made with a Diplodia found fruiting on a dead branch of pomelo bearing many pycnidia of *Sphaeropsis tumefaciens*. (See inoculation No. 121 (pomelo), July 29, 1909, lime strain, p. 58.) The effects produced by the two fungi are very similar and resemble the black-rot recently described by Pole Evans,[1] except that in the latter the lemons become distinctly sticky and there is a greenish brown exudate wherever they come in contact with anything, phenomena wholly lacking in the inoculations previously described.

*Inoculations on apples.*—Rather green apples were inoculated by inserting bits of mycelium or spores under the skin (both lime and orange strains were used). They were then placed under a dry bell jar. In four days there was a brown rotted area 2 to 2.5 cm. in diameter. At this stage the softening did not extend beyond the discoloration, but subsequently it advanced more rapidly than the browning. In eight days the apples were softened throughout, but, with the exception of one which was just beginning to wither slightly, they had retained their shape perfectly. In 12 days mature pycnidia were present on the apple inoculated with the fruiting orange strain. The epidermis finally became almost entirely black and somewhat shriveled. Seventeen days after inoculation many of the brown septate bodies (see p. 16) were observed. (Pl. VIII, fig. 5.) These were much less common three weeks later. (Under observation five weeks.)

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[1] Evans, I. B. Pole. On the Structure and Life History of Diplodia Natalensis N. Sp. Science Bulletin No. 4, Transvaal Dept. of Agriculture, 1910.

In another set of inoculations the apples, though soft-rotted throughout, did not begin to shrivel for over two weeks. Although pycnidia were present in great numbers only a few contained spores. These apples were under observation nearly four months.

Apples inoculated with the Diplodia (see inoculations on lemons) rotted much more quickly, but otherwise behaved similarly to the above. This Diplodia resembles *Diplodia natalensis* very closely, but the spores are wholly unlike those of *Sphaeropsis tumefaciens,* even when immature.

*Inoculations on pomelos.*—Inoculations were also made on very young green pomelos, about 2.5 cm. in diameter, still on the trees. The culture used was a 9-day-old potato culture which was fruiting abundantly. There were no signs of disease in three months.

## SUMMARY.

1. From knots on lime and orange trees received from Jamaica a fungus, *Sphaeropsis tumefaciens* Hedges, has been isolated which reproduces the knots when sound trees are inoculated with pure cultures of it. Small knots have also been obtained on pomelo, lemon, *Citrus trifoliata,* and tangerine. From these artificially produced knots the fungus has been again isolated and with it successful inoculations on sound trees have been made.

2. The fungus penetrates the tissues far beyond the original point of infection (see description of Tree II, inoculations of December 1, 1904, p. 42) and a badly infected tree is a mass of tumors, sometimes so close together that one can not put a finger between them. On the other hand, there may be considerable distance between two secondary tumors (1½ feet in one case observed, see p. 42) with no external evidence of the fungus, but microscopical examination reveals its presence in small quantities throughout the intervening portion of the stem. This important fact should be borne in mind in pruning for the purpose of eradicating the disease.

3. An abnormal number of new shoots often grow from the knots, forming a witches'-broom. This is characteristic of the disease under favorable conditions.

4. Ultimately that portion of the branch above the knot dies.

5. The mycelium may occur in any or all of the tissues of the stem, producing black areas if present in abundance.

6. Pycnidia may or may not be produced. The Jamaican lime strain has, up to this time, fruited rarely, pycnidia having been observed only in a few very old cultures, and a small number of artificial infections on the hosts (lime, pomelo, and *Citrus trifoliata*). A vigorously

fruiting orange strain has arisen, however, from cultures grown from germinating spores in plates poured from pycnidia in a 6-months-old corn-meal culture. Another orange strain isolated later from the same lot of knots has never, to the writers' knowledge, produced pycnidia in culture media, although it has produced them on lime shoots (see inoculations of July 29, 1909, p. 58). A second vigorously fruiting orange strain has been isolated from one of the knots produced by the inoculations of July 20, 1910.

7. Spermagonia occur both on the host and in cultures.

8. Chlamydospores are often present in abundance in old cultures, but have never been observed on the host.

9. No perithecia or conidia have ever been observed.

10. A stroma is produced on many media but not upon the host.

11. The fungus is easily isolated by ordinary mycological methods and grows well on a large variety of media.

12. The fungus liquefies gelatin, partially or entirely digests casein (see milk cultures, p. 32), and has a strong diastasic action. Optimum temperature about 30° C.

13. The fungus can live for some years in the host. It has been isolated from secondary knots four years and eight months after the tree was inoculated and one year after it has been cut down and brought into the laboratory.

14. Affected limbs should be removed, care being taken to cut them off well below the lowest knot (see "Summary," par. 2). Badly diseased trees should be rooted out and burned, that they may not prove a source of infection to sound trees through the dissemination of the fungous spores. Great care should be taken to select only sound trees for grafting purposes. The recent discovery of this or a similar disease in Florida should put growers in the United States on their guard against the spread in this country of a new menace to citrus fruits.

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